EFFECT OF AMINO ACID BALANCE ON ENERGY AND NITROGEN METABOLISM IN GROWING BROILER CHICKENS

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Thesis submitted for the degree of Doctor of Philosophy

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2004

DECLARATION

I hereby declare that I have written this thesis based on my own work, and the contribution of others has been clearly indicated. This thesis has not been submitted elsewhere for any other degree or professional qualification.

Ji-Hyuk Kim

ABSTRACT

Three experiments were performed to test the assumption that imbalanced dietary amino acid mixtures must lead to increased heat production (HP). The first experiment was based on diets formulated to have a wide range of crude protein (CP) concentrations but a fixed concentration of lysine, formulated to be the first-limiting amino acid. In the second (converse) experiment, lysine concentration was varied over a wide range while CP content was kept constant. To prevent the masking of dietary effects by thermoregulatory demands, the third experiment was performed at 30 °C with the diets similar to the diets used in the second experiment. The detailed relationships among amino acid balance, nitrogen (N) metabolism and energy (E) metabolism were investigated in a computer-controlled chamber calorimetry system. The results of experiments were compared with the predictions of a computerised simulation model of E metabolism.

In experiment 1, with constant lysine and varying CP, there was a 75% increase in N intake as CP concentration increased. This led to a 150% increase in N excretion, with no significant change in HP. Simulated HP agreed with the empirically determined results in not showing a trend with dietary CP. In experiment 2, with varying lysine but constant CP, there was a 3-fold difference in daily weight gain between the lowest and highest lysine diets. HP per bird increased significantly with dietary lysine concentration. There was still an effect when HP was adjusted for body weight differences, but it failed to maintain statistical significance. Simulated HP results agreed in showing little effect of varying lysine concentration and growth rate on HP. Based on the results of these two experiments, the third experiment was designed to test the response of birds to dietary lysine in high ambient temperature. In experiment 3 which performed at high ambient temperature (30 °C), HP per bird increased significantly with dietary lysine content, whether or not adjusted for body-weight. The trend was greater than in the previous experiment (20 °C).

To investigate the effect of amino acid balance and protein quality on growth rate and carcass characteristics, growth trial experiment was performed on a larger scale. Four diets varying CP contents were used. The results showed that there was no significant effect of high protein diet on growth rate and carcass characteristics.

A free-choice feeding experiment was also performed to investigate the sensitivity of the bird to its diet on the basis of amino acid balance, especially related to lysine concentration. Over the whole period of the experiment, birds consumed some of each diet offered, but preference was shown for the moderate or high lysine diet over the low lysine diet. Growth rate reflected total lysine intake. The birds offered the choice between Ideal Protein diet and High Protein diet consumed about 2.5 times as much of the Ideal protein diet as of the imbalanced diet.

ACKNOWLEDGEMENTS

I am extremely grateful to my supervisor, Dr Murdo MacLeod for his guidance, supervision and encouragement throughout my study. Special thanks for his kind help and patience from the beginning of my study until writing this thesis. Without him all of this could not be possible. I am also grateful to my university supervisor, Dr Neil Jessop for his advice and support during the study.

I would like to thank Professor Colin Whittemore for his valuable comments, advice and encouragement for my thesis. I also would like to thank Dr Jai-Jun Choung and Korean Collaboration Centre for financial support for my PhD study. I also wish to thank Professor Chang-Won Kang of Kon-Kuk University for his advice and encouragement to continue my study in the UK.

I would like to thank Mrs Lindsey McNeill, Mrs Kim Bernard and Mrs Audrey Laurie for their great help and excellent laboratory assistance throughout my experiment. I also would like to thank the Librarians of Roslin Institute for their help in providing me with reference books and papers. I give my thanks to Mr David Waddington for his advice for the experimental design and statistical analysis of my experiments.

I wish to thank the pastors and congregation of Edinburgh Korean Church for their kind support and their precious prayer for me and my family.

Finally, my very special thanks must be offered to my parents for their constant trust, support and prayer. I am also deeply grateful to my dear wife who helped me with love and encouragement. I dedicate this work to them.

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Chapter 1

INTRODUCTION

1.1. General Introduction

As poultry industries throughout the world increase in size, the importance of feed production also continues to increase. One of the main objectives of animal nutrition is to formulate diets that allow a predetermined rate of production to be achieved at least cost. The supply of balanced nutrients in animal feed is one of the most critical requirements for achieving optimal growth. Protein, especially, is the major nutrient that determines the performance of animals, assuming that energy is non-limiting. It is also an expensive dietary constituent and there is much concern about optimizing its dietary concentration in commercial practice. Dietary protein must be judged as a source of individual amino acids which are needed for synthesis of protein by the animal.

Ever since Almquist (1947) pointed out the importance of the ratio of the individual amino acids in the diet, many scientists have shown that dietary proteins which do not contain adequate amounts of essential amino acids to satisfy the animal's requirement cannot be used efficiently for their growth and maintenance and eventually cause growth failure and deficiency symptoms. For these reasons, the terms 'protein quality' and 'ideal protein' have been introduced.

Protein quality also influences the environment, because of nitrogen excretion. One of the ways of minimizing nitrogen excretion in the waste is through diet

composition, rather than dealing with it after the waste has been produced. Reduction in nitrogen excretion and improvement in the efficiency of nitrogen deposition can be obtained by matching the amino acid composition of the diet with the amino acid requirements of the bird for maintenance and production. This is a protein blend which comes as close as possible to providing amino acids in the exact ratios required by the bird. Therefore, it is environmentally and sometimes economically beneficial to provide an ideal blend of protein in diets.

Additionally, excretion of nitrogen from excess amino acids can cause welfare problems; carcase defects such as breast blister and hock burn are often attributable to high uric acid excretion rates caused by poor quality protein. Breast blister can also be exacerbated by poor feather growth due to amino acid deficiency.

Energy is another major cost factor of the poultry diet and as a consequence has been the subject of considerable research. Energy content of the diet should be seriously considered and is used as a basis to determine most nutrient concentrations because most animals tend to eat to satisfy their energy requirements assuming that the diet is adequate in essential nutrients. Poultry can control their energy intake over a range of energy: protein ratios. Energy: protein ratio also affects the growth and body composition. It is, therefore, very important to know to what extent the relationship between energy and dietary protein influences the bird's performance.

Although there has been much discussion and hypothesis about the effect of protein quality on energy metabolism and growth in birds, relatively few experimental data exist. The conventional expectation is that an imbalanced blend of amino acids must lead to increased energetic costs of catabolism and excretion, accompanied by

reduced food conversion efficiency. MacLeod (1997) found, however, that heat production was closely correlated with rate of protein accretion, which in turn was more strongly associated with the intake of the first-limiting amino acid (lysine) than with total protein intake. The experiments described in this thesis were, therefore, performed to investigate the detailed relationships among amino acid balance, nitrogen utilisation and energy metabolism using a calorimetry system. The experimental diets were formulated to have a wide range of protein concentrations or lysine concentrations. The effect of protein quality on growth rate and carcase characteristics were also examined in a large number of birds to compare with calorimetry results. Additionally, a free choice feeding experiment was performed to examine the bird's ability to select for protein quality.

1.2. Literature Review

1.2.1. Energy metabolism

1.2.1.1. *Energy terminology*

Energy is not a nutrient but is rather a product of energy-yielding nutrients, resulting from the oxidation of organic molecules ingested and absorbed from the diet or from metabolism of energy stored in the form of protein, fat or carbohydrates. In animal nutrition, energy is, quantitatively, the most important item in the diet because food intake is strongly influenced by the energy content of the diet and all animal feeding standards are based on energy needs.

Gross energy (GE) is heat energy resulting from complete oxidation of food or other substances. GE is measured in a bomb calorimeter. GE values, by themselves, are not sufficient for evaluating food for animal use since animals cannot digest some components, especially fibre, as completely as the heat of combustion might indicate. Some food is undigested resulting in a loss of energy as faeces. Faeces also contain material, originating from the body, that has been abraded or secreted into the alimentary tract. The difference between the energy content of the food and the energy content of the faeces is therefore termed the Digestible Energy (DE) of the food. Cellulose and other vegetable matter can be converted into digestible end-product by bacterial growth in the gut of most herbivorous animals. This results in loss of energy in the form of the waste product methane. The remaining part of the digestible energy represents nutrients which are absorbed into the blood stream, and waste products from their further metabolism are lost as the energy excreted in urine.

The remaining energy is the metabolisable energy (ME) of the food. Birds excrete urine and faeces together via the cloaca. Therefore, it is difficult to measure digestibility and DE values are not generally used in poultry feed formulation. For poultry, the gaseous products are usually negligible so that ME represents the gross energy of the food minus gross energy of excreta. A correction for nitrogen retained in the body is frequently applied to yield an ME_N value (Anderson *et al.*, 1958). ME_N is the most common measure of available energy in poultry nutrition.

Sibbald (1976) developed a procedure for estimating the true ME (TME) of diets for birds. In this procedure, a bird is fasted, weighed and tube-fed a weighed amount of the diet or feedstuff in question. The bird is returned to its cage and droppings for the next 24 h are collected and analysed for components of interest. This method appears to give a more accurate measure of ME. The formula for calculation is : TME intake(I_{TME}) = I_E – ((faecal+urinary)energy + (endogenous (faecal + urinary) energy), where I_E is gross energy intake. Endogenous energy was measured by analysing the excretion after 24 h fasting. True metabolisable energy (TME) is GE of the food minus the GE of the excreta of food origin. In other words, TME is ME corrected for endogenous energy losses.

Net energy (NE) is equal to ME minus the heat increment and heat of fermentation. Heat increment can be defined as the heat production associated with nutrient digestion and metabolism over and above that produced prior to food ingestion. There has been a debate on the choice of ME or NE for energy evaluation of poultry feedstuffs. Some scientists compared these two energy system (Halnan, 1951; Hill and Anderson, 1958; De Groote, 1974) and this subject was recently reviewed by Pirgozliev and Rose (1999). Since NE accounts for more of the losses in metabolism

of food or by the animal than other measures described, it should be the method of choice for food evaluation or standard of animal requirements. In poultry nutrition, however, the ME system is still more widely used because NE varies with environmental temperatures outside the comfort zone and because of the limited amount of data on feedstuffs and birds.

1.2.1.2. *Measurement of heat production and energy metabolism*

The activity of feeding and the metabolism caused by digestion and assimilation of food increase an animal's heat production. To study the utilisation of ME, it is necessary to measure either the heat production of the animal or energy retained in the tissues, that used for productive work, or in a product. In cases where we know one of these quantities, then the other can be determined by subtracting the known one from ME. Heat production can be measured in various ways. There have been many reviews about the techniques used for measurement of energy metabolism in farm animals and humans (*e.g.* McLean and Tobin, 1987; Blaxter 1989; McDonald *et al.*, 1995). The principles are relatively simple and have changed little. However, there has been much progress in terms of technology for more precise and less labour intensive measurements.

Measurement of heat production can be obtained by calorimeter. The heat may be measured directly by physical methods or it may be inferred from quantitative measurement of some of the chemical by-products of metabolism. Direct calorimeters measure heat produced by the animal within them directly, using thermocouples or changes in the amount of heat produced in cooling the chamber. These types of calorimeters are quite expensive to build and to operate due to their complexity and are seldom used, especially for large animals.

Indirect calorimetry estimates heat production from quantitative measurements of oxygen consumption and carbon dioxide production during metabolism. Brouwer (1957, 1965) published an equation for calculating heat production from respiratory exchange.

HP (MJ) = 16.18 O_2 (l) + 5.16 CO_2 (l) – 5.93 UN (g) – 2.42 CH_4 (l)

UN : urinary nitrogen.

The open-circuit chamber method was one of the earliest types of calorimeters, having been devised by Petenkoffer and Voit in 1875 (cited by McLean and Tobin, 1987), although at that time only the volume of air and its carbon dioxide content could be measured. There have been a large number of reports in the literature of open-circuit chambers. Jequier and Schutz (1983) have described an open-circuit chamber for human studies; open-circuit chambers have been combined with direct calorimetry for studies on man (Dauncey *et al.*, 1978) and primates (Dale *et al.*, 1967). Open-circuit chambers have also been widely used with poultry (Bonsdorff Petersen, 1967; Misson, 1974), pigs, sheep and cattle (Verstegen *et al.*, 1971; Vermorel *et al.*, 1973; Young *et al.*, 1975). A multi-chamber system for poultry has been built at the Roslin Institute, Scotland. This system has been described in detail by Lundy *et al.* (1978) and MacLeod *et al.* (1985).

An open-circuit calorimeter is an air-ventilated box containing the bird under investigation. The oxygen and carbon dioxide concentrations of fresh air entering and sample gas leaving the calorimeter and the flow-rate at which sample gas is withdrawn are measured at frequent, regular time intervals. Oxygen consumption is calculated from the difference between oxygen flow-rate into and out of the calorimeter. A continuous correction is made for changes in oxygen concentration in the calorimeter during the period of observation. Carbon dioxide production over the

same period is calculated by analogous methods to those used for calculating oxygen consumption. Urinary nitrogen excretion can be determined by chemical analysis of cloacal excreta.

Thus in calorimetric experiment under laboratory conditions the retention of energy in the body can be estimated, not directly, but as a difference between measurements of the intake of energy as food, and the sum of energy excretions and the heat produced (Blaxter, 1989).

1.2.1.3. *Heat production and body size*

In the nutritional physiology of animals, it is important to compare the information obtained from calorimetric studies with more normal environmental conditions. Calorimetric data are obtained under very specific conditions, and it is not feasible to attempt to duplicate all of the situations which are encountered by animals in their normal environment or to account for differences in age, size and species. In order to compare heat production between individual animals within or between species, the conditions under which the measurement are made must be standardised.

Early nutritional research showed that heat production was not directly correlated to body weight of animals and much effort was expended to develop means of predicting heat production and establish some overall law that applies to animals in general. In 1883, Rubner showed that the fasting metabolism of seven dogs, varying in body weight from 3.2 to 31.2 kg, was approximately constant when expressed per unit area of body surface (m^2). Voit (1901) published a table which showed that the fasting metabolisms of a number of different species were also proportional to their surface areas (cited by Blaxter, 1989). It has since been shown that the rate of

cooling is proportional to surface area, which varies with the square of linear size or to the 2/3 power of weight if specific gravity is constant. Surface area, therefore, varies with the square of linear size or the 2/3 power of volume, so heat production can be related to body surface or volume. Despite the fact that surface area of a living animal is not constant due to various factors, it can be related reasonably well to surface area estimated by multiplying body weight by a fractional power. This is referred to as metabolic weight or metabolic size. There has been extensive controversy on this subject in the past. Kleiber (1932) and Brody and Proctor (1932) showed that when metabolism was related directly to body weight metabolism was proportional to a power of weight higher than 2/3 (cited by Blaxter, 1989). Brody and Proctor found the power to be 0.734 and Kleiber estimated it to be 0.75. Kleiber (1961) stated that the slope as estimated from his original set of data was 0.739. In both sets of data errors were such that proportionality to the power 0.75 can be accepted as a valid estimate (Blaxter, 1989).

1.2.1.4. *Basal and fasting metabolism*

The minimal energy expended to maintain respiration, protein turnover and other vital metabolic process of a resting, thermally neutral and post-absorptive state is known as the basal metabolic rate (BMR). The post-absorptive state is used so that the heat increment or heat of fermentation do not add to the body heat production. Animals cannot be controlled to keep their body at complete rest in post-absorptive state at a certain period of day. Therefore, the fasting metabolic rate is measured in domestic animals. Generally, smaller omnivorous animals reach the post-absorptive state earlier than ruminants. Mitchell (1962) stated that chickens reach this state in about 48 h and ruminants in 3-5 d.

The bird's calculated or measured BMR does not equal its daily basal requirement for dietary ME because BMR measurements are determined in fasted birds. Conversion of BMR into a basal ME requirement requires the addition of the amount of energy needed for the heat increment related to food consumption.

1.2.2. Amino acid metabolism

1.2.2.1. *Protein quality and amino acid balance*

The dietary protein requirement is actually a requirement for the amino acids contained in the protein. Amino acids are organic compounds containing both an amine group and a carboxylic acid part. It is in the form of the constituent amino acids that protein is absorbed from the intestine. However, not all amino acids within intact proteins need to be released as such during digestion, because there is some absorption of peptides. Uptake of free amino acids as peptides may be advantageous to the birds, relative to processing of free amino acids from within the gut lumen. Boorman and Ellis (1996) suggest that one advantage of a bird utilising peptides is that there will be less bacterial degradation within the digesta and that this activity should not be underestimated.

Some of the amino acids are essential in that they have to be provided by the dietary protein or as supplements in the diet. Some amino acids can be produced within the body of the birds by transformation of other amino acids. Therefore, protein requirements have two components. Firstly, the essential amino acids needed by the birds because it cannot synthesize them, or synthesize them rapidly enough. Secondly, sufficient protein to supply either the non-essential amino acids themselves or to supply amino nitrogen for their synthesis.

The protein quality refers to the assortment and proportions of amino acid: the more complete the assortment and the more nearly the proportions approach the physiological needs of animals for amino acid, the higher the quality of protein (Lloyd *et al.*, 1978). However, the amino acid composition of a foodstuff is not the

final measurement for protein quality because some of the amino acids may not be biologically available to animals. We also have to consider a reasonable ratio of essential amino acids to non-essential amino acids. Overall, the protein quality of diet is a function of its quantity, its digestibility and amino acid balance. Boorman (1999) stated that relative to the animal's requirements one essential amino acid is always likely to be in shorter supply than the others. It is referred to as the first limiting amino acid.

In recent decades, there have been numerous reports studying amino acid requirements of poultry from scientific and economic aspects. The reported values differ widely because the amino acid requirements are affected by various factors. Ishibashi (1990) summarized that the factors are classified into four categories, environmental factors, genetic background, condition of animals and dietary factors. In the dietary factors, various factors are involved such as dietary metabolisable energy, availability of dietary amino acids, antagonism and imbalance among amino acids, deficiency and excess of amino acids, palatability, and dietary crude protein or metabolically related amino acid levels. The statement of a requirement is the rate at which a bird needs to be supplied with energy or a nutrient in order to carry out a set of functions such as maintenance, protein growth, or egg production at certain rates (Emmans and Fisher, 1986).

There have been many reports suggesting the requirements or recommendations for crude protein content and individual amino acid concentrations. Table 1.1 shows crude protein and amino acid requirement of broilers published by the National Research Council (NRC, 1994). Although recommendations by various researchers differ to some degree, NRC recommendations have been used as a standard for

formulating poultry diets for many years. However, slightly higher concentrations of some amino acids are being used in practice for better productivity. For example, dietary lysine concentration can have a large influence on breast meat yield since breeders have selected the broiler for increased meat yield, breast meat represents a large portion of total carcase meat, and breast muscle contains a high concentration of lysine (Kerr *et al.*, 1999). Kerr *et al.* (1999) reported that breast meat weight and proportional yield were significantly increased by increasing dietary lysine up to 121% of NRC recommendation. Leclercq's review (1998) also suggested that lysine exerts specific effects on body composition at dietary levels higher than that required for maximum growth rate, which also results in an improved feed conversion ratio. Wallis (1999) found that dietary supplements of methionine increased breast meat yield and decreased abdominal fat in growing broilers.

Table 1.1 *Amino acid requirement of broilers as percentages or units per kilogram of diet (90% dry matter) by NRC (1994)*

	0-3 weeks [a]; 13.4 [b]	3-6 weeks [a]; 13.4 [b]	6-8 weeks [a]; 13.4 [b]
Crude protein [c]	23.00	20.00	18.00
Arginine	1.25	1.10	1.00
Glycine + serine	1.25	1.14	0.97
Histidine	0.35	0.32	0.27
Isoleucine	0.80	0.73	0.62
Leucine	1.20	1.09	0.93
Lysine	1.10	1.00	0.85
Methionine	0.50	0.38	0.32
Methionine + cystine	0.90	0.72	0.60
Phenylalanine	0.72	0.65	0.56
Phenylalanine + tyrosine	1.34	1.22	1.04
Proline	0.60	0.55	0.46
Threonine	0.80	0.74	0.68
Tryptophan	0.20	0.18	0.16
Valine	0.90	0.82	0.70

a. The 0- to 3-, 3- to 6-, and 6- to 8-week intervals for nutrient requirements are based on chronology for which research data were available; however, these nutrient requirements are often implemented at younger age intervals or on a weight-of-feed consumed basis.

b. These are typical dietary energy concentrations, expressed in MJ MEn/kg diet which is equal to 3200 kcal /kg.

c. Broiler chickens do not have a requirement for crude protein *per se*. There, however, should be sufficient crude protein to ensure an adequate nitrogen supply for synthesis of non-essential amino acids. Suggested requirements for crude protein are typical of those derived with corn-soybean meal diets, and levels can be reduced when synthetic amino acids are used.

Adapted from Nutrient Requirements of Poultry, NRC (1994)

A protein which has a balance of amino acids that exactly matches a bird's requirement, along with sufficient non-essential amino acid nitrogen to permit the synthesis of all of the non-essential amino acids, is referred to as an 'ideal protein' (Baker and Han, 1994 a; Cole and van Lunen, 1994).

Considerable attention has been given to the assessment of amino acid requirements of broiler chickens and to the definition of optimal dietary amino acid balance. Grau (1948; cited by Morris *et al.*, 1999) stated that the requirement for an amino acid expressed as a proportion of the diet increases in direct proportion to the protein content. He reported that the lysine requirement in chicks fed on diets based on sesame meal formulated to supply 50 to 300 g CP/kg diet was higher in the 300 g CP/kg diet than in the 200 g CP/kg diet, even though the growth rate achieved with an optimal supplement of lysine was essentially the same at these two protein levels. Further research confirmed this principle. Nelson *et al.* (1960) reported that the requirement for methionine + cystine was a constant proportion of the protein in diets containing from 212 to 275 g CP/kg. Boomgaardt and Baker (1971, 1973) reported that lysine and tryptophan requirements for maximum growth are constant proportions of the dietary protein content ranging from 140 to 230 g CP/kg diet. Robbins (1987) reported similar results for threonine. More recent reports (*e.g.* Mendonca and Jensen, 1989; Barbour *et al.*, 1993; Morris *et al.*, 1987, 1999) have dealt with the relationship between dietary protein content and amino acid requirement and all of these authors have concluded that the requirement increases as a linear function of dietary protein content.

1.2.2.2. *Imbalancing effect and amino acid utilisation*

Theoretically, an animal's response to dietary protein is a response to the most limiting amino acid in that protein and therefore it should be possible to achieve the same maximum response with any protein, irrespective of its quality, provided sufficient protein is fed (Boorman and Ellis, 1996). Carpenter and de Muelenaere (1965) firstly tested this hypothesis by feeding young chickens on groundnut flour supplemented with lysine to ensure sulphur-containing amino acid deficiency and cautiously concluded that under certain conditions, higher levels of poor quality protein will result in nearly as good growth as can be obtained with practical diets containing good quality protein. However, Wethli *et al.* (1975) reported that maximum growth could not be obtained when groundnut meal or soybean meal was used as a simple supplement to a cereal-based diet, even though very high dietary protein levels were used. They stated that 'the only explanation which fits the evidence is that the amino acids supplied by these low-quality proteins are in such disproportion ... that the utilization of the first limiting amino acid(s) is impaired'.

At very high concentrations of dietary protein, the excess load of absorbed amino acids may exert an imbalancing effect, requiring an increased supply of the first limiting amino acid if maximum growth is to be achieved. This effect of excess amino acids has been demonstrated many times, using free amino acids. The term imbalance has been defined by Harper (1964) who investigated this effect using growing rats. Harper and Rogers (1965) stated that dietary amino acid imbalance precipitates its adverse effects by reducing food intake while efficiency of amino acid utilisation remains unimpaired. The issue of amino acid imbalance assumed practical significance in poultry nutrition with studies of Wethli *et al.* (1975). They invoked this phenomenon to explain the inferior utilization of the first limiting amino acid in

low quality protein sources. Some reports (Morris *et al.*, 1987; Abebe and Morris, 1990, Morris and Abebe, 1990; Mendonca and Jensen, 1989) indicated that imbalance may increase the requirement of the chick for the first limiting amino acid, but this effect has not been corroborated by D'Mello (1990). Most experiments with imbalancing mixtures of amino acids report a depression in food intake but show no reduction in utilization of the first limiting amino acid when growth rate is regressed on amino acid intake (Fisher *et al.*, 1960; Harper *et al.*, 1970; Boorman and Ellis, 1996).

There is good quantitative evidence for the interdependence of lysine and arginine and of valine, leucine and isoleucine in chick diet (D'Mello and Lewis, 1970). Antagonism usually occurs because of interactions between structurally similar amino acids. Lysine specifically antagonises the utilisation of arginine. Leucine impairs the utilisation of isoleucine and valine; these three compounds are branched-chain amino acids. In practice, the antagonisms can be reduced by supplementation of the diet with arginine, isoleucine or valine. Since their main excretory product is uric acid, poultry cannot synthesise arginine. This makes them particularly sensitive to lysine antagonism. There is increased activity of kidney arginase in chicks receiving excess lysine, leading to increased arginine breakdown (D'Mello, 1994).

As mentioned before, much research has shown that, when diets varying in CP were fed to chicks, the amount of lysine needed to maximise growth rate at each protein concentration was a fixed proportion of the protein. This proportionality rule is well established for diets with limiting protein contents and can be explained by the hypothesis that, as the supply of a first-limiting amino acid is increased, the limit to response is determined by the supply of the second-limiting amino acid. It is

important, however, to recognize that such statements may not be true in situations of amino acid oversupply and/or poor amino acid balance.

Fisher *et al.* (1960) used sesame meal as the sole source of protein in the diet, varying the protein and lysine concentrations and the degree of imbalance by adding a mixture of amino acids lacking lysine. They observed the typical effects of imbalance such as low food intake and growth rate on the more imbalanced diets, especially at low protein concentrations. Netke *et al.* (1969) using purified diets limiting in lysine, isoleucine or leucine emphatically confirmed the conclusion of Fisher *et al.* (1960) and this interpretation of the effects of imbalance was accepted by Harper *et al.* (1970) in their comprehensive review of the subject.

D'Mello (1990) compared lysine responses in three diets, one containing 225 g CP/kg and two others containing 315 g CP/kg, obtained by adding unbalanced mixtures of free amino acids. The results showed no reduction in lysine utilisation in the presence of excess amino acids. However, the lysine additions to the 'high protein diets' were not sufficient to yield an estimate of optimum response.

Boorman and Ellis (1996) performed the experiment with protein mixtures of different quality by mixing maize gluten meal and soyabean protein concentrate in constant proportions, supplementing with tryptophan, threonine and arginine to adequacy and varying amino acid score by varying additions of free lysine. They reported that there is no evidence, on deficient intakes, that the utilisation of the limiting amino acid is affected adversely by poor protein quality (amino acid imbalance) and it is not possible to elicit maximum response to the limiting amino acid by feeding large amounts of poor-quality protein. MacLeod (1997) stated that

the strongly negative correlation between protein retention per g of lysine consumed and lysine: CP ratio may have resulted from a greater catabolism of lysine when it is present in greater concentration relative to other amino acids.

Overall, it appears unlikely that amino acid imbalance is a satisfactory explanation for the protein effect on lysine utilization and the issue raised by the data of Morris *et al.*(1987) and others (Mendonca and Jensen, 1989; Abebe and Morris, 1990) remain essentially unresolved.

1.2.2.3. *Ideal amino acid profile*

Amino acid requirements of animals depend on various factors such as sex, strain, diet, body composition and environment. In swine nutrition, researchers have been using ideal ratio (to lysine) for different weight categories as a basis for formulating swine diets to solve this problem (Wang and Fuller, 1989; Chung and Baker, 1992). This method is based on the fact that, although the amino acid requirements change because of the factors mentioned above, the ideal ratio of essential amino acids to lysine will be only marginally affected within a certain age range. The benefit of applying the ideal amino acid pattern is that once an ideal ratio of essential amino acids to lysine is established for a certain age range, one can concentrate on determining the lysine requirement accurately under a variety of conditions and can calculate the requirement for all other indispensable amino acids by applying their ideal ratio to lysine (Mack *et al.*, 1999). This concept in poultry is supported by work reported by Morris *et al.* (1987) where it was found that for chickens over the range of 140 to 280 g CP/kg, lysine requirement could be expressed as a constant proportion of the protein (5.4%). Other reports (*e.g.* Boomgardt and Baker, 1973; Robbins, 1987) also confirmed this hypothesis. However this may not be true in

cases where there are excesses of amino acid and/or poor amino acid balance (Cole and van Lunen, 1994). Amino acid profile, therefore, should be considered assuming that appropriate levels of high-quality protein are being used.

According to Baker and Han (1994a), lysine was selected as the reference amino acid for three primary reasons: 1) its analysis in feedstuffs, unlike those of tryptophan and sulphur amino acids, is relatively simple and straightforward; 2) a considerable body of data exists for digestible lysine needs of poultry; and 3) unlike several other amino acids (*e.g.* methionine, cystine and tryptophan), absorbed lysine is used only for protein accretion.

Tables 1.2 and 1.3. show ideal amino acid patterns for broilers aged 0 to 3 wk and 3 to 6 wk, respectively, calculated as a percentage of lysine from various studies (NRC, 1994; Han and Baker, 1994; Rhone-Poulenc Nutrition Guide, 1993; Uzu, 1993; Mack *et al.*, 1999). After 3 wk old, the ratio for some amino acids such as sulphur amino acids, threonine and tryptophan have to be increased because of changing maintenance requirements.

Table 1.2 *Amino acid profiles expressed as percentage of lysine for broiler chickens 0-3 wk old by some research groups*

	NRC [1] (Total AA)	RPAN [2] (Total AA)	RPAN [2] (Digestible AA)	Baker & Han [3] (Digestible AA)
Lysine	100	100	100	100
Methionine	46	47	51	36
Arginine	113	110	117	105
Valine	82	83	79	77
Threonine	73	64	65	67
Tryptophan	18	19	19	16
Isoleucine	73	75	78	67
Histidine	32	-	-	32
Leucine	109	140	150	109

1. National Research Council (1994)
2. Rhone-Poulenc Animal Nutrition (1993)
3. Baker and Han (1994)

Table 1.3 *Amino acid profiles expressed as percentage of lysine for broiler chickens 3-6 wk old by some research groups*

	Baker & Han [1] (Digestible)	RPAN [2] (Digestible)	Mack *et al.* [3] (Digestible)
Lysine	100	100	100
M+C	75	81	75
Methionine	37	48	-
Arginine	105	108	112
Valine	77	85	81
Threonine	70	67	63
Tryptophan	17	19	19
Isoleucine	67	75	71
Histidine	32	-	-
Leucine	109	144	-

1. Baker and Han (1994)
2. RPAN Rhone-Poulenc Animal Nutrition (1993)
3. Mack *et al.*(1999)

Ideal amino acid patterns for laying hens and broiler chickens are shown in Tables 1.4 and 1.5. It appears that the ideal amino acid pattern for laying hens is slightly different from broilers. The laying hen has been selected for high egg production and low muscle mass while the broiler has been selected for rapid weight gain and high muscle mass. This may explain the differences in pattern between these two types during growth; a greater proportion of dietary amino acids is required for muscle production in the broiler chickens. When the layer reaches its laying period,

amino acid requirements are mainly for maintenance and egg production. Since maintenance has a relatively small amino acid requirement compare to egg production, the ideal amino acid pattern will be governed to a large extent by the requirement for egg production. Table 1.6 shows the amino acid composition of protein in poultry carcase, feather and egg. The amino acid composition of carcase and egg is similar due to the similarity in the balance of amino acids in tissues and eggs. Consequently, the ideal balance of amino acids required for growth and for egg production are similar.

Table 1.4 *Amino acid profile and requirement for broiler calculated from NRC (1994)*

	0-3 wk		3-6 wk		6-8 wk	
	Profile	% of diet	Profile	% of diet	Profile	% of diet
Lysine	100	1.1	100	1.0	100	0.93
Arginine	114	1.25	110	1.1	118	1.0
Histidine	32	0.35	32	0.32	32	0.27
Isoleucine	73	0.8	73	0.73	73	0.62
Leucine	109	1.2	109	1.09	109	0.93
M+C	82	0.9	72	0.72	71	0.6
Methionine	45	0.5	38	0.38	38	0.32
Threonine	73	0.8	74	0.74	80	0.68
Tryptophan	18	0.2	18	0.18	19	0.16
Valine	82	0.9	82	0.82	82	0.7

Table 1.5 *Amino acid profile and requirement for layer calculated from NRC (1994)*

	0-6 wk		6-12 wk		12-18 wk		18-laying	
	profile	% of diet	profile	%	Profile	%	profile	%
Lysine	100	0.85	100	0.6	100	0.45	100	0.52
Arginine	118	1.0	138	0.83	149	0.67	144	0.75
Histidine	31	0.26	43	0.26	38	0.17	38	0.2
Isoleucine	71	0.6	83	0.5	89	0.4	87	0.45
Leucine	129	1.1	142	0.85	156	0.7	154	0.8
M+C	73	0.62	87	0.52	93	0.42	90	0.47
Methionine	35	0.3	42	0.25	44	0.2	42	0.22
Threonine	80	0.68	95	0.57	82	0.37	90	0.47
Tryptophan	20	0.17	23	0.14	24	0.11	23	0.12
Valine	73	0.62	87	0.52	91	0.41	88	0.46

Table 1.6 *Amino acid composition (g/kg protein) of protein in poultry carcase, feathers and egg*

	Carcase	Feather	Egg
alanine	69[c]	35[c]	54[a]
arginine	69[cd]	61[c]	68[a]
asparagine	0	68	0
aspartic acid	93[c]	62[c]	107[a]
cystine	11[bc]	67[c]	18[a]
glutamic acid	136[c]	87[c]	120[a]
glutamine	0	67	0
glycine	82[c]	68[c]	62[a]
histidine	29[bcd]	8[cde]	24[a]
hydroxyproline	20	20	0
isoleucine	43[bd]	50[de]	56[a]
leucine	73[bc]	81[de]	83[a]
lysine	80[bd]	20[de]	62[a]
methionine	27[bd]	7[de]	32[a]
phenylalanine	41[bcd]	50[de]	51[a]
proline	61[c]	92[c]	38[a]
serine	44[c]	91[c]	78[a]
threonine	42[bc]	48[de]	51[a]
tryptophan	10[b]	7[d]	18[a]
tyrosine	30[bcd]	27[de]	40[a]
valine	47[bcd]	76[de]	75[a]

[a] Lunven *et al.*(1973) , [b] Hakansson, *et al.* (1978), [c] Nitsan *et al.* (1981), [d] Hurwitz *et al.* (1983), [e] Blair,*et al.* (1981)

1.2.2.4. *Low protein diets and nitrogen utilisation*

It is generally recognized that reducing the dietary protein level is the most efficient way to lower nitrogen pollution. As mentioned in the general introduction, the reduction of nitrogen excretion and efficiency of nitrogen deposition can be controlled by matching the amino acid composition of the diet with the amino acid requirement of the bird for growth, maintenance and production.

In some regions, environmental pollution places a severe limitation on the expansion of animal agriculture, because of the implications for the contamination of soil and water. Growing pigs, for example, excrete approximately 70% of the nitrogen present in their feed and broilers excrete 58% (Table 1.7). This shows that a substantial proportion of the consumed nitrogen is excreted in faeces and urine.

Table 1.7 *Fate of nitrogen consumed in the feed by different livestock*

	Feed Protein (g/kg DM)	Body (%)	Faeces (%)	Urine (%)
Beef cattle	150	22	30	48
Piglet	184	40	10	50
Growing pig	170	32	15	53
Laying hen	170	32	12	56
Broiler	217	42	10	48

Adapted from Tamminga (1992)

Diets can be formulated with a reduced protein content and higher concentration of amino acid supplements, providing the appropriate concentrations of essential amino acids while avoiding large excesses. This is a sound approach to minimizing nitrogen input and utilising dietary nitrogen efficiently. Use of supplemental amino acids allows the total dietary protein to be reduced while still meeting the bird's needs. Estimates of the reduction in nitrogen excretion possible with laying hens range from 20% (Blair *et al.*, 1976) to over 50% (Summers, 1993). For broilers, the estimates range from 10% (Han *et al.*, 1992) to 30% (Parr and Summer, 1991). Blair *et al.* (1999) showed results that reduction in dietary CP content caused a 10-27% reduction in the total amount of nitrogen excreted during the 6-week broiler rearing period and with layers, there was a 30-35% reduction in daily N output.

The availability of commercial synthetic amino acids allows the use of low protein diets to reduce pollution by avoiding an excess of each amino acid above its requirement. Low protein diets have several additional advantages. Keshavarz (1991) postulated that low protein diets increase the tolerance of birds to elevated environmental temperatures. Furthermore, the kidneys are exposed to lower stress in eliminating the end products of nitrogen metabolism and reduced nitrogen excretion lowers the ammonia build-up in poultry house.

It is important to determine the ideal balance between amino acids in order to maximize the performance level and minimize the nitrogen excretion, especially on a low protein diet. There is conflicting evidence as to whether low protein diets supplemented with amino acids can support maximum growth rate. Edmonds *et al.* (1985) demonstrated an inferior performance of chicks fed an amino acid-supplemented, 16% protein diet in comparison with those fed 24% protein diet.

Fancher and Jensen (1989) and Mendonca and Jensen (1989) also reported an inferior performance for birds fed low protein, amino acid-supplemented diets compared with performance of birds fed a more conventional protein concentration. Fancher and Jensen (1989) stated that chicks fed the diet containing 17.8% protein supplemented with synthetic amino acids to meet the requirements suggested by NRC (1984) were significantly lower in body weight gain and feed efficiency than chicks fed the diet with 24.5% protein. More scientists (Uzu, 1982; Pinchasov *et al.*, 1990; Holsheimer and Janssen, 1991; Moran *et al.*, 1992) claimed that maximum performance cannot be achieved by feeding broilers low protein diets supplemented with amino acids.

However, other scientists have achieved performance of broiler chickens fed low protein, amino acid-supplemented diets equivalent to that of birds receiving higher protein diets. Twining *et al.* (1974) reported that broiler chicks receiving a low protein starter diet had lower body weights and food conversions at 4 week-old compared to those receiving the required amount. However, when they subsequently consumed an adequate finisher diet, they showed compensatory growth, with final performance almost equal to controls. Although compensatory gain can restore performances to those obtained under optimal circumstances, possible carcase quality alterations such as increased body fat and reduced protein content may occur (Moran, 1979). Lipstein *et al.* (1975) also reported similar results with low protein diet fed to birds 5 to 9 wk of age. Schutte (1987) fed diets containing either 20 or 16% CP to chicks from 7 to 28 days of age and obtained equal weight gain and feed efficiency with 16% protein diet supplemented with all essential amino acids equivalent to the level present in the 20% protein diet. Parr and Summers (1991) and Stilborn and Waldroup (1988, 1989) also obtained optimal

performance with low protein diets supplemented with amino acids. But, like Summers and Leeson (1985), Parr and Summers (1991) found that carcase fat increased with low protein diets. Deschepper and De Groote (1995) reported that it is possible to obtain the same performances with low protein diets supplemented with synthetic amino acids, using an ideal amino acid balance. These authors concluded that broilers overeat in a compensatory attempt to obtain the limiting amino acid required for optimal growth rate, and this results in increased carcase fat deposition. Therefore, the research on methods to reduce carcase fat invites attention, because of the need to produce lean meat. It has been demonstrated that it is possible to reduce carcase fat by restricting feed intake (Jackson *et al.*, 1982). Uzi (1983) stated that addition of non-essential synthetic amino acids such as glutamic acid reduces the carcase fat content of broilers fed on low protein diet supplemented with amino acids. Fancher and Jensen (1989) reported that the addition of glutamic acid to a low protein diet significantly reduced abdominal fat deposition in male broilers.

1.2.2.5. *Amino acid balance and welfare problems*

With each moult the protein in the old feathers is lost and new feather growth requires a supply of amino acids. Each successive juvenile feather coat is heavier than the previous one and will therefore require an increased input of amino acids.

Dietary amino acids play a critical role in feather development since 89-97% of the feather dry matter is protein (Fisher *et al.*, 1981). Cystine and methionine, the sulphur-containing amino acids, are the major amino acids involved in the synthesis of feather keratin (Wheeler and Latshaw, 1981). Cystine and methionine are required for general maintenance and growth in addition to their role in feather

synthesis. The relative proportion of the sulphur containing amino acids is much greater in the integument than in muscle tissue and marginal dietary deficiencies of these amino acids will often be initially manifested as abnormal feathering (Deschutter and Leeson, 1986). Cystine and methionine are frequently the first limiting dietary amino acids and accurate determinations of the sulphur containing amino acid requirements during various stages of growth, therefore, are necessary to ensure optimum body and feather growth.

The sulphur amino acids are the most important for feather growth, although feathers showing signs of amino acid deficiency have been noted in chicks fed rations deficient in arginine, valine, leucine, isoleucine, phenylalanine and tyrosine (Anderson and Warnick, 1967). Although amino acid deficiency can be indicated by abnormal feathering, antagonisms or imbalances of various amino acids can often result in similar abnormalities of plumage (Deschutter and Leeson, 1986).

Birds on wet litter have a high incidence of breast skin lesions and breast blisters, with the breast area caked with litter and faeces (Greene *et al.*, 1985; Gonder, 1987). The excretion of excess nitrogen caused by amino acid imbalance may influence breast blister formation because of the high uric acid excretion rates produced by poor quality protein.

1.2.3. Energy metabolism and amino acids

1.2.3.1. *Energy utilisation and its prediction*

The dietary requirement for carbohydrates and fats is less exacting than those for proteins. Fats and carbohydrates are the main energy sources in the diet (see Figure 1.1). Protein, or rather the amino acids that constitute protein, can also be used as an energy source, but this only occurs in situations in which it is present in excess or imbalance, or where there is a shortage of fat and/or carbohydrates. The typical energy yields of these major nutrients, as determined by bomb calorimetry, are 18.8 kJ/g for carbohydrates, 23.5 kJ/g for protein and 38.4 kJ/g for fat (Blaxter, 1989). The actual amount of energy obtainable from these nutrients by birds is somewhat less, as this would require each of the nutrients to be absorbed completely by the gut. Both fat and carbohydrates can be fully oxidised in the body to CO_2 and water. But the nitrogen of protein is not released in its fully oxidised state since most is excreted as uric acid. This also reduces the energy available from protein to 17.99 kJ/g.

There are no specific storage protein molecules except skeletal muscle protein which is the largest potential reservoir of amino acids for egg protein during the laying period. When dietary protein is either scarce or absent, endogenous protein, particularly from skeletal muscle, is degraded to generate a source of amino acids. When the protein is provided in excess, it cannot be stored and becomes degraded and deaminated, providing carbon skeletons for biosynthesis of fats and carbohydrates. In birds, the surplus nitrogen is excreted mainly as uric acid rather than urea or ammonia. The reason is considered to be an adaptation to the incubation requirements of the egg. During embryonic development, waste uric acid precipitates out of solution, preventing the osmotic imbalance that would occur if

urea or ammonia were excreted. This osmotic advantage may also aid in water conservation in adult birds (Klasing, 1998)

Amino acids are absorbed by active transport processes involved with carrier proteins. Four carrier mechanisms were identified catering for the neutral amino acids, the basic amino acids, for dicarboxylic acid and for proline, hydroxyproline and glycine (Blaxter, 1989). These transport mechanisms, called symports, need energy. Similar active transport and diffusion process coupled to sodium ion movement apply to carbohydrate absorption. Glucose and galactose are absorbed by active transport and diffusion process while fructose absorption is passive. The heat production which accompanies active absorption arises from the ATP-dependent sodium transport component; it is the enthalpy change associated with oxidations which leads to ATP formation – the ultimate source of heat.

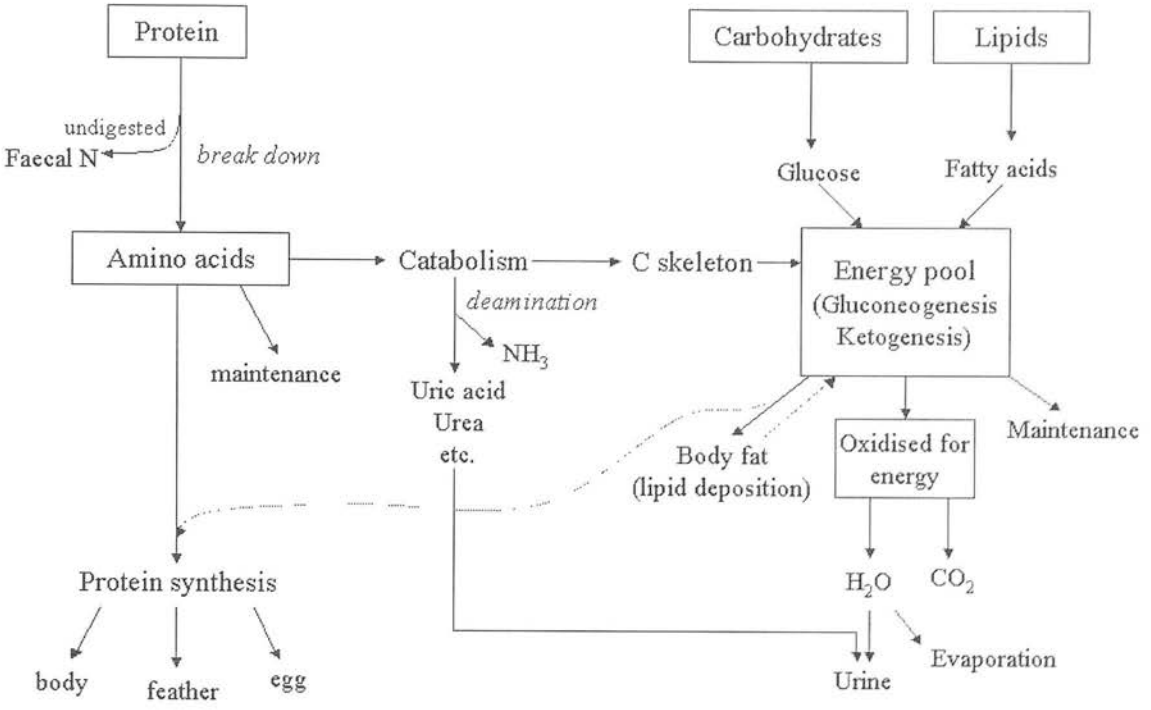


Figure 1.1 *Diagram illustrating the metabolism of energy-yielding nutrients in the body*

To predict the energy value of the diet or its chemical constituents, researchers *(e.g.* Schulz, 1975, 1978; Livesey, 1984, 1985; MacLeod, 1994, 1998) have been working on modeling using the equations of the major biochemical pathways in terms of ATP generation and utilization. Livesey (1984) calculated energy yield as ATP from carbohydrates, fats and proteins which have been absorbed and are available for cellular catabolism. His model treats all substances purely as energy sources and therefore corresponds with an ME form of evaluation.

In MacLeod's model (1994, see Figure 1.2), the stoichiometric foundation for biochemically-based simulation of energy metabolism was derived largely from

Schulz (1978). But different stoichiometric coefficients were used for amino acid break down due to the differences between mammalian and avian amino acid metabolism (MacLeod, 2000). Amino acid compositions of proteins in body, feather and egg were compiled from various sources.

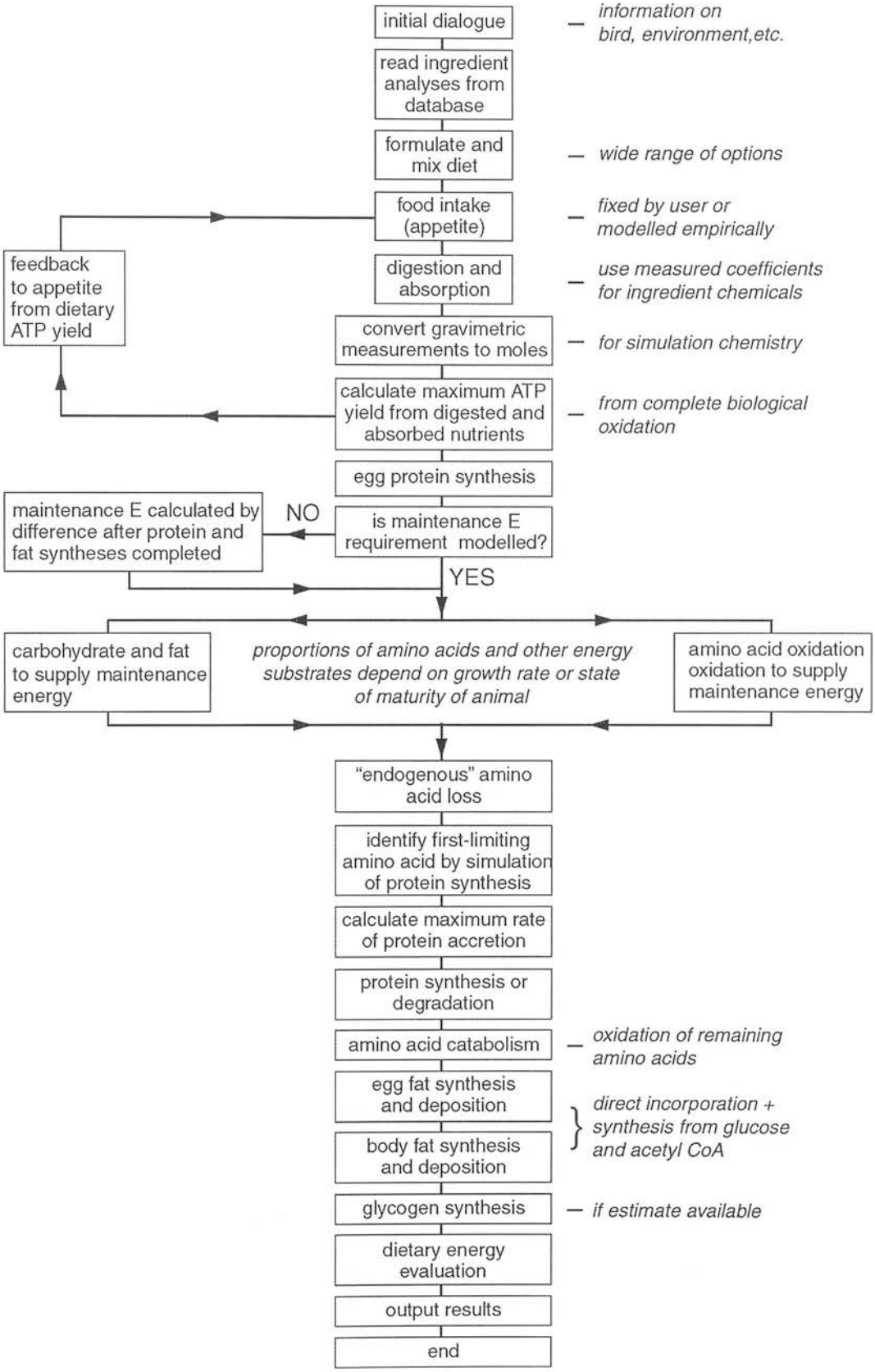


Figure 1.2 *Flow diagram of the simulation model of MacLeod (1994)*

Emmans (1984, 1994) reported a method for estimating the "effective energy" of diets or ingredients, in which ME is adjusted for the heat increment of feeding by applying linear coefficients to five measurable components of the interaction between the animal and its diet.

Nitrogen-corrected ME is estimated as :

$$ME_n \text{ (kJ/d)} = ME - a\ (6.25\ NR)$$

where NR is nitrogen retention (g/d).

According to the principle of the conservation of energy, the ME yielded from the diet will either be retained in the body or lost as heat. In other than the short run all the energy retained by the animal can be taken to be in the form of the potential energy of either protein or lipid (carbohydrate deposition is negligible in the long term). The equation follows that :

$$ME_n \text{ (kJ/d)} = (h_p - a) \times PR + h_l \times LR + H$$

where PR and LR are the rates of retention of protein and lipid (g/d), h_p and h_l are the heats of combustion of protein (23.8 kJ/g) and lipid (39.6 kJ/g), and H is heat production (kJ/d). Although poultry excrete uric acid rather than urea, the trans-species nitrogen correction (a) of 5.63 kJ/g of protein retained (35.2 kJ/g of N) is close to the 36.5 kJ/g of N conventionally used for poultry (MacLeod, 2000).

When the protein and fat retention is known, ME requirement can be calculated by prediction of heat production. Most of Emmans (1994) is concerned with this. Heat production has two components; fasting heat production (FHP) and heat increment of feeding (HIF). FHP comes from the catabolism of the protein and lipid. FHP is given by :

$$FHP \text{ (kJ/d)} = (h_p - a) \times PR + h_l \times LR$$

where PR and LR are the rates of loss while fasting considered as positive quantities.

Maintenance heat production (MH) is calculated with the simplifying assumption that only lipids are catabolised in the body when fasted :

$$ME\ (kJ/d) = FHP - w_u \times FUN$$

where w_u (kJ/g of N) is the heat production associated with the synthesis and excretion of urinary N, and FUN is urinary nitrogen excretion (g/d) during fasting.

Heat increment of maintenance (HIM, kJ/d), ignoring methane production in the case of poultry, is given by the equation :

$$HIM\ (kJ/d) = w_d \times FOM + w_u \times UN$$

where w_d (kJ/g) is heat production associated with the production of faecal organic matter, FOM (g/d). Maintenance ME requirement (MEM, kJ/d) is given by :

$$MEM\ (kJ/d) = MH + HIM$$

For a diet leading to positive retention of both protein and lipid, PR and LR will be related to the production of FOM and UN (ignoring methane production in poultry). Heat increment of feeding is, therefore, given by :

$$HIF\ (kJ/d) = w_d \times FOM + w_u \times UN + w_p \times PR - w_l \times LR$$

where w_p and w_l are the heat productions associated with protein and lipid deposition, respectively.

And ME (kJ/d) need by animal is given by :

$$ME\ (kJ/d) = ER + MH + HIF$$

where ER is energy retention.

1.2.3.2. *Heat increment and amino acids*

Ingestion of food increases both heat production and the energy retention in the body. A fed animal produces more heat than a fasted one. There have been a number of research studies that try to explain the increased HP after food intake. Several general theories explaining the heat increment have been produced. One of these older theories which is still valid is that of Rubner (1902, cited by Blaxter, 1989). He ascribed the specific dynamic action (SDA) to the waste heat produced by reactions necessary to support the physiological process of the body. All energy sources fed to an animal will increase heat production. But the increase caused by protein or amino acids has stimulated most research interest. The study of Rubner demonstrated that protein caused a larger increase in heat production than either carbohydrates or fat.

When fed as a high proportion of the diet, protein is a less efficient source of energy than carbohydrates or fat (Blaxter, 1989). In cases when protein is used as an energy source for maintenance or production, nitrogen must be excreted. Dietary protein also increases HP resulting from nitrogen excretion and HP associated with net synthesis of product. Additional effects of dietary amino acids on HP beyond this level and HP for the production would also be increased by protein because dietary protein stimulates protein turnover (Reeds *et al.*, 1982; Reeds and Fuller, 1983). The energy cost of protein accretion has been estimated as 44 kJ ME/g (ARC, 1981). Of this amount, 23.7 kJ can be recovered by combustion of the protein. The cost of synthesis is, therefore, 20.3 kJ ME/g. A stoichiometric estimate of the cost of protein synthesis is 3.45 kJ ME/g (Millward *et al.*, 1976). The difference of approximately 17 kJ ME/g protein is probably a consequence of protein-stimulated catabolism and turnover (Musharaf and Latshaw, 1999).

The results from many studies suggest that higher dietary protein will reduce energetic efficiency. The study of Rubner showed that the SDA of protein was expressed more markedly at high temperatures than low temperatures. The use of more synthetic amino acids and less intact protein permits essential amino acid needs to be met at lower concentration of dietary protein (Keshavarz and Jackson, 1992).

1.2.3.3. *Energy metabolism and amino acid balance*

In the series of experiment by MacLeod (1990, 1991, 1992), it was found that large differences in energy: protein ratio were accommodated largely by changes in body composition, with no indication of regulatory changes in heat production (diet-induced thermogenesis). This was shown with high energy and low protein diets and by both chamber calorimetry and comparative-slaughter techniques. The conventional expectation has been that surplus amino acids which are not needed for protein synthesis must stimulate an additional increase in metabolic rate and lead to increased energetic costs of catabolism and excretion. The hypothesis from several studies (*e.g.* Guillaume and Summers, 1970; Okumura and Mori, 1979; Tasaki *et al.*, 1970, 1976) has been that, when protein synthesis becomes limited by the first-limiting essential amino acid, amino acids present in excess of the resulting requirement enter the pool of substrates available as energy sources. It implies that this would result in a greater heat increment than would occur with a balanced amino acid mixture. Baldini (1961) found that heat production was increased in methionine deficiency diet. Guillaume and Summers (1970) also reported the increased heat production in diet containing methionine-cystine adequacy with other amino acids in relative excess. However, it can also be argued that the catabolism of surplus amino acids would, to some degree, spare the utilisation of other substrates for heat

production, without necessarily having a large effect on total metabolic rate (MacLeod, 1997).

MacLeod's experiment (1997) examined three dietary factors which have been implicated in elevation of metabolic rate: high energy concentration, low protein concentration and imbalanced amino acid mixture. High energy concentration and low protein concentration can be summarised in terms of CP:TME ratio. Since the diets were formulated with lysine as the first-limiting amino acid, protein quality can be summarised as lysine:CP ratio. It was found that differences in energy expenditure could be explained almost entirely (93%) by differences in quantities, and therefore costs, of protein and fat accretion. There was no indication of regulatory diet-induced thermogenesis. Heat production was not significantly correlated with CP:TME ratio and was negatively correlated with dietary TME concentration. Heat production was closely correlated with rate of protein accretion, which in turn was more strongly associated with intake of the first-limiting amino acid (lysine) than with total protein intake. Heat production on an imbalanced, lysine-limited, amino acid mixture was no greater than on a balanced amino acid source with the same lysine concentration.

Boorman and Ellis (1996) found no adverse effect of protein quality on lysine utilisation and detected some indication of increasing net utilisation with decreased protein quality. That there was no limitation of nitrogen retention by another amino acid on the better balanced diets (high lysine: CP ratio) was reinforced by the similarity between nitrogen retentions on these diets and those of Boorman and Ellis (1996). A possible explanation may be that, at the extreme protein imbalances of the present study, the amino acid pool was catabolised according to molar abundance

(MacLeod, 1997). The smaller the proportion of lysine in the dietary amino acid pool the smaller may have been the proportion lost to catabolism; the excess non-limiting amino acids may thus have had a sparing effect. This effect will be maximal when CP:TME ratio is greater, since more protein must function as an energy source (MacLeod, 1990). However, a complicating factor is that the diets giving the lowest values for CP retained per g of lysine consumed were also those with the greatest proportion of lysine as supplemental lysine-HCl. The utilisation of crystalline lysine would have had to be extremely low for this to have accounted for the observed effect. There are indications of a similar effect in the re-analysis by Abebe and Morris (1990) of experiments by Morris *et al.* (1987); the lysine requirement for 0.97 of maximum growth rate (estimated from the Reading model) decreased from 56.4 to 44.5 g lysine/kg CP as dietary CP increased from 140 to 280 g/kg.

There is evidence that supplementation with synthetic amino acids to avoid increasing the total protein concentration of the diet leads to greater fat deposition in commercial broilers (Deschepper and De Groote, 1995). This may occur because there is less surplus protein to be metabolised, but a more important cause may be the converse; that a greater proportion of dietary energy must be consumed as carbohydrate and fat, which are deposited more efficiently as body fat than excess amino acids would be.

1.2.3.4. *Energy requirement and its response in body composition*

Energy content is widely acknowledged to exert a dominant role in the regulation of feed intake in growing poultry. Boomgaardt and Baker (1973) examined the effects of dietary ME concentration on the response of chicks to graded doses of methionine + cystine. Their results showed three distinct growth response curves for the three

dietary ME concentrations; 10.9, 12.6 and 14.2 MJ/kg. However, the efficiency of utilisation of these amino acids is unaffected by energy content of the diet since a single response curve is obtained on plotting weight gain against methionine + cystine intake. D'Mello (1979) stated that dietary ME, within the range tested, exerts its effect principally through variations in feed intake and without affecting amino acid utilisation.

In chicks, which are usually fed *ad libitum*, the energy: protein ratio has been seen as important because the ME content of the diet determines feed intake (if other nutrients are non-limiting) and thus influences protein intake (Morris *et al.*, 1999). Edward and Campbell (1991) emphasised the importance of dietary energy in determining the limit of the response to protein in the pig. D'Mello (1993) referred to reduced energy yield and amino acid utilisation, but was not entirely convinced by the hypothesis. It should also be noted that MacLeod and Boyd (1994) reported that heat production was no greater from birds receiving lysine from a poor-quality protein source than from birds receiving the same dietary lysine concentration from a balanced protein mixture.

Broiler chicks have traditionally been fed relatively high-energy diets to promote efficient feed utilisation and maximise growth rate (Leeson and Summers, 1991). More recently, lower-energy content diets have been tried in an attempt to resolve such problems as ascites (Leeson *et al.*, 1995), and it is now realized that overall growth rate is little affected. Therefore, the broiler chick may adapt to diets of low energy content, and simply eat more feed in an attempt to maintain energy intake, much the same as does the Leghorn (Payne, 1967). Leeson *et al.* (1996) proved that the broiler has a good ability to control its feed intake based on desire to normalize

energy intake and as energy intake is decreased, or there is increased protein intake, the bird deposits less carcase fat.

Energetic efficiency of ME use for tissue gain is dependent upon many variables. Such efficiency has been shown to vary with substrate source for lipogenesis at approximately 75, 84 and 61% for carbohydrates, fats and proteins respectively (De Groote, 1969; Chudy and Schiemann, 1971; Hoffmann and Schiemann, 1971). The high availability of fat ME for tissue gain, however, is dependent upon the fat being used for lipogenesis (Bossard and Combs, 1961). Utilisation of protein for tissue energy gain would be expected to be dependent upon the biological value of the protein source and should not be constant (De Groote, 1973). Indeed, one could summarize that the bird's energetic efficiency for protein or any substrate is the net result of partitioning consumed substrate energy into maintenance needs as well as protein and fat accretion.

Studies directed towards partitioning consumed ME into retained energy and heat production have not produced results that are consistent with either the energetic efficiencies discussed above or among themselves. Though proteins reportedly have the lowest efficiency of ME use for tissue gain, HP is elevated when birds are fed diets containing marginal crude protein levels (Davidson *et al.*, 1964). In contrast, MacLeod (1990, 1992) reported that though low dietary energy: protein ratios were negatively correlated with fat deposition, the effect was independent of energy dissipation as heat. However, in these studies, the basal diet composition changed with treatment which confounded consumption patterns for carbohydrate, protein and fat.

1.2.4. Diet selection

Most wild animals are able to select a diet suited to their needs by choosing between the wide variety of feedstuffs available to them. They also have the ability to learn to select a diet that avoids deficiencies or toxic excesses. It is of evolutionary advantage to the animal living under natural condition. However, this will vary according to the physiological state of the animal, the climate, the ease of obtaining food and the extent of the social competition (Forbes, 1995). Under natural conditions, wild animals meet different foods which vary widely in nutrient composition depending on source, season and stage of maturity. From these they are capable of selecting a diet which is adequate for their requirements (Summers and Leeson, 1979). Domestic animals and poultry have demonstrated this ability to select and regulate their diet to meet their requirements for growth, maintenance and production (Hughes, 1984; Rose and Kyriazakis, 1991).

In the early part of the 20th century, Kempster (1916) and Rugg (1925) observed that free-choice laying fowl produced more eggs than those fed a single food and that Leghorns could balance their own diets (cited by Forbes and Shariatmadari, 1994). Since Grahame (1932) and Funk (1932) reported that birds can select a balanced diet from several imbalanced diets, free-choice feeding has received much attention. Studying diet selection is important to understand the nutrient requirements of animals both from scientific and commercial points of view. There is much evidence from several species of an ability to select an adequate diet when offered choices between diets varying in energy and protein.

To predict the amount of food that chickens will consume when given free access to a diet we have to consider what Emmans (1981) termed 'Rules' for controlling food intake. It is believed that chickens eat primarily to satisfy their needs for energy (Hill and Dansky, 1954; Morris, 1968; Scott *et al.*, 1969). This concept has been given credibility due to experiments which show that diluting foods with an inert material leads to a constant intake of the food and increased intake of the filler. Emmans (1981) noted that growing chickens fed on maize starch are unlikely to eat as much energy as others fed on a balanced chicken diet. This, and the observation that chickens get much fatter on protein deficient diets, led to his proposal that animals seek to eat for the first limiting resource. The observed increase in intake with marginally deficient diets tends to confirm this proposition.

Most of the raw materials used in animal diets have concentrations of available energy within a fairly narrow range, from 9 to 13 MJ/kg DM, whereas the content of nutrients such as protein varies over a much wider range (Forbes, 1995). An animal can control its energy intake by varying the amount of DM it consumes but can then only control its intake of nutrients independently of energy if it has access to two or more foods which differ in content of the nutrient in question. Because of its quantitive importance, high cost and the polluting nature of its excretory wastes, protein has been widely studied in terms of diet selection. When choice diets provided more or less than the optimum concentration of protein, the preferred diet of growing pigs was that which allowed growth at a rate similar to those fed a single diet that was assumed to be optimal (Kyriazakis *et al.*, 1990). Holcombe *et al.* (1976) and Shariatmadari and Forbes (1993) have shown that layer chickens were able to choose the optimum diet to meet their requirements between different diets varying in their protein concentration. Leeson and Summers (1978) found that fast

growing male turkeys selected a high protein diet rather than a low protein diet although males and females chose a diet with a similar energy concentration. Experiments have indicated that chicks also have shown the ability to adjust food intake to compensate for calcium deficiency (Holcombe *et al.*, 1975; Hughes and Wood-Gush, 1971a) and thiamine deficiency but not a sodium deficiency (Hughes and Wood-Gush, 1971b). Broilers also showed similar differences in diet selection between sexes (Rose and Lambie, 1986). Picard *et al.* (1993) showed that young chickens distinguished between a balanced diet and one deficient in certain amino acids. Newman and Sands (1983) reported that broiler chicks chose an adequate lysine diet rather than a low or zero lysine diet.

Emmans (1977) suggested that self-selected diets which reflect the nutritional requirements of the bird would have appreciable economic benefits by avoiding excessive intakes of expensive components of the diet. Cumming (1984, 1994) also stated that choice feeding can be viewed as a flexible feeding technique that is able to meet the wide range of individual needs within the flock under different environmetal conditions, while having both practical and economic advantages.

According to Forbes (1995), there are two prerequisites for diet selection. One is the need for sensory differentiation. He stated that it is necessary for animals to be able to differentiate between two or more foods if they are to select proportions in order to make up a balanced diet. If the nutrient in question does not differ markedly or is only required in trace amounts, and colourless, it is necessary to give a cue by means, for example, of artificial flavouring and/or colouring. Secondly, in order for an appetite for a nutrient to develop it is necessary for animals to learn to associate the sensory properties of each food with its content of the nutrient in question. They

take advantage of any nutritious foods which may become newly available (Stephens and Krebs, 1986).

1.3. Summary of literature review

Evaluation of energy in the diet is very important in animal nutrition because food intake is strongly influenced by the energy content of the diet. This means that the intake of other nutrients, such as amino acids, is affected by their ratio to energy content. The activity of feeding and the metabolism caused by digestion and assimilation of food increase the animal's heat production. Heat production can be measured by calorimetry.

Dietary protein should be judged as a source of individual amino acids which are needed to satisfy the amimal's requirements for growth and metabolism. An animal's response to protein is a response to the most limiting amino acid in that protein. The concept of protein quality or ideal protein was established to achieve the maximal performance of the animal with minimal excretion of nitrogen.

Theoretically, surplus amino acids which are not needed for protein synthesis stimulate an additional increase in metabolic rate and lead to increased energetic costs of catabolism and excretion. However, it has sometimes been shown that there was no measurable diet-induced thermoregulatory effect when an imbalanced amino acid mixture was fed.

According to research in choice-feeding, it seems that the birds, including poultry, have an ability to choose the appropriate diet in relation to essential amino acids. This information can be used to assist in formulating the optimal diet.

Chapter 2

GENERAL MATERIALS AND METHODS

2.1. Experimental animals

All experiments were performed with male broiler chickens. Chicks (1d old) from the same commercial line (Ross Breeders Ltd.) were obtained in batches and reared on a common diet in brooders until they were transferred to the experimental location. The experiments had an acclimatization period of 7 days in which animals were allowed to become accustomed to the experimental diet and chambers or cages.

2.2. The calorimetry system

2.2.1. System structure

The indirect open-circuit calorimetry system has been described by Lundy *et al.* (1978) and improvements in the gas analysis system by MacLeod *et al.* (1985). It consists of three main parts : animal chambers, the analysis system and a computer-based control and data-acquisition system.

The chamber (Figure 2.1 and 2.2) is designed to hold a bird or group of birds of total weight up to 4 kg. The chamber is an airtight, thermally insulated, climatically controlled Perspex box, of internal dimensions 600 mm × 600 mm × 450mm high, fitted with a Perspex door which is sealed by a rubber gasket and release nuts. A partition from floor to ceiling, separated by about 50 mm from the front and rear

walls, divides the chamber into a test section (a) and an air-conditioning system (b). A 13-W fluorescent light is placed in the insulation board over the midline of the test section. The test section is floored by a shallow tray that supports a heavy plastic-covered wire mesh. The tray is used for the quantitative collection of excreta. A calibrated drinker designed to minimize evaporative water losses is provided in the test section. The test section also has a holder for a feeding trough to prevent the feeder from being turned over. The air conditioning system occupies about one-third of the chamber's volume, and contains an orifice plate (1), recirculating fans (2), two heat exchangers (3), humidifying tray (4) and two heaters (5).

The analysis system is situated in a controlled-temperature room kept at 20 °C. It consists of three principal parts: a gas volume flowmeter, a paramagnetic oxygen analyser and an infra-red carbon dioxide analyser. Flow rate is determined by measuring the pressure drop across a sintered bronze disc. Partial pressure of oxygen is measured by a Servomex OA 184 paramagnetic oxygen analyser. Partial pressure of carbon dioxide is measured by a Gascard II infra-red gas analyser. Analysis system pressure and flow are servo-controlled. Figure 2.4 shows the electronic control of sample-gas flow rate and absolute sample-gas pressure improved by MacLeod *et al.* (1985).

The computer control and acquisition system controls the selection of different samples (fresh air, gas sample) and sends the chosen sample to the analysers (O_2 and CO_2 analysers). Also, this system acquires data from the measuring transducers and calculates oxygen consumption, carbon dioxide production and finally heat production. The functional relationship between these sections is shown in Figure 2.3.

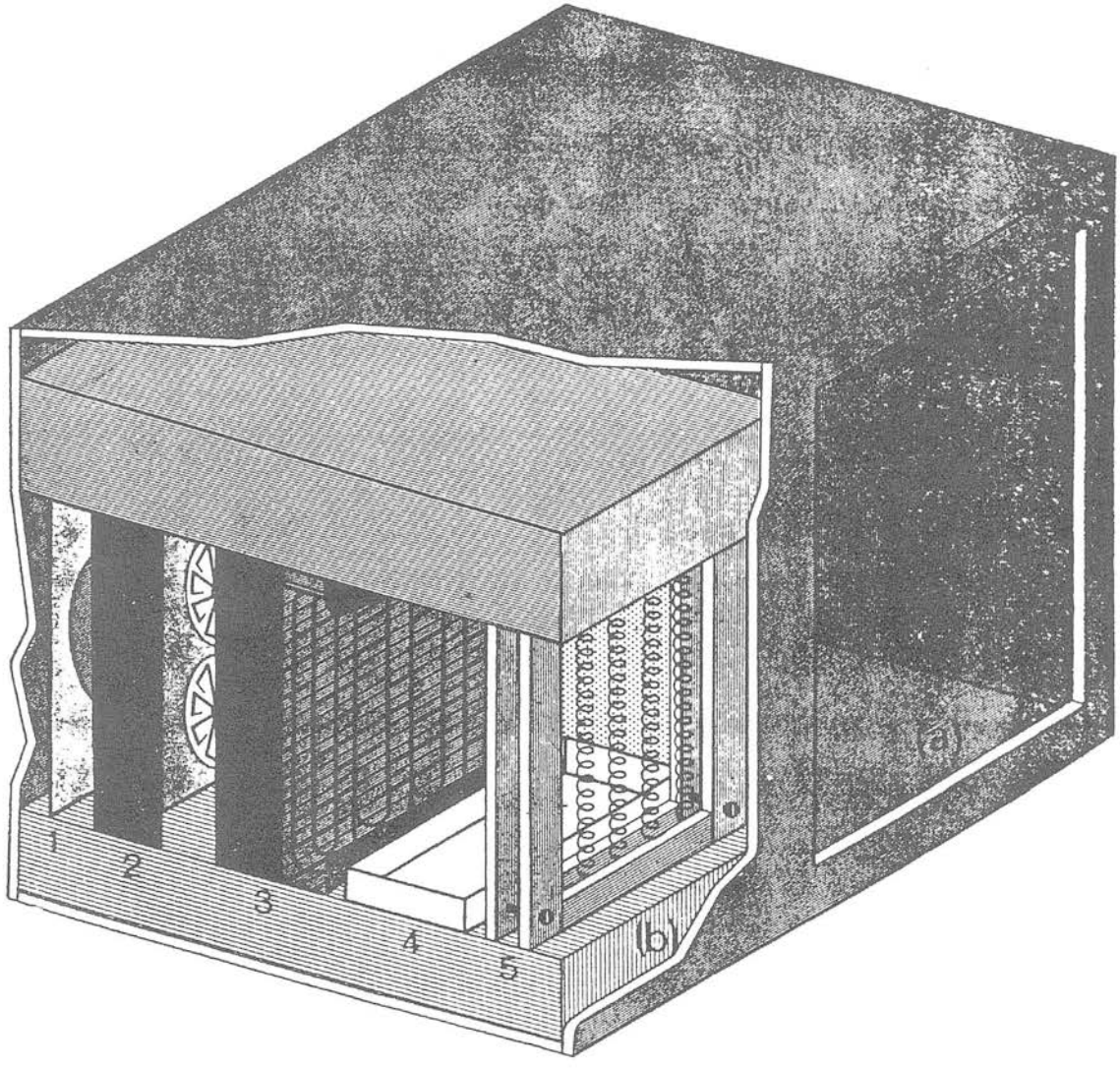


Figure 2.1 *Block diagram of calorimetry chamber (Lundy et al., 1978)*

(a) test section (b) air conditioning system

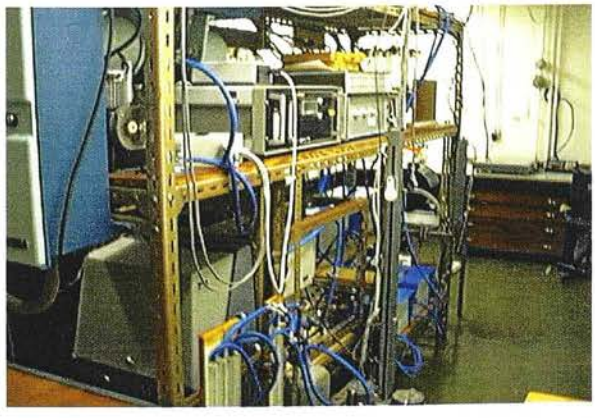
1. orifice plate 2. recirculating system 3. heat exchanger

4. humidifying tray 5. heating coil

(a) Multi-calorimetry chambers

(b) Birds allocated in pairs

(c) Control and data acquisition system



(d) Analysis and calibration system

Figure 2.2 *Multi-calorimeter chamber system*

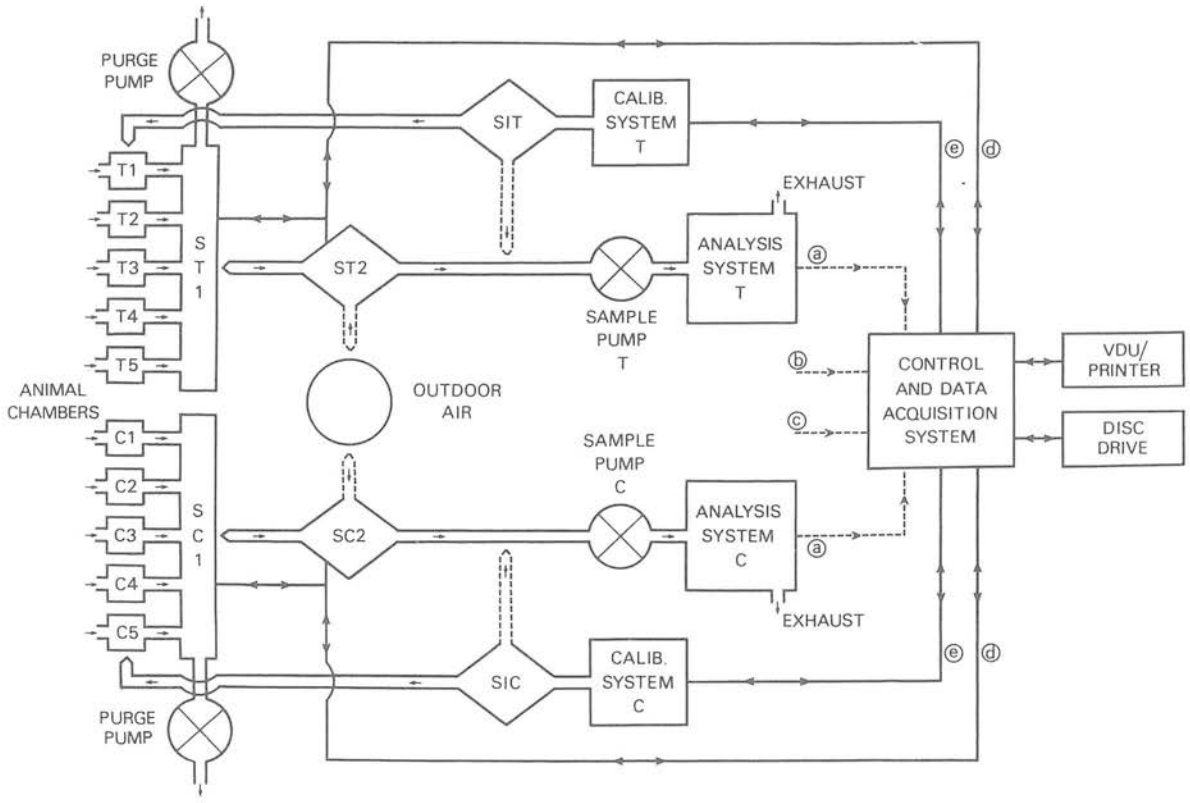


Figure 2.3 *Gas and information flow in the multi-calorimeter system (MacLeod et al., 1985)*

ST1, ST2 computer-controlled switch valve 1 and 2 of the turkey system

SC1, SC2 computer-controlled switch valve 1 and 2 of the chicken system

SIT, SIC calibration gas injection switch valves

T, C turkey and chicken system

Broken and intact lines indicate alternative switch positions

(a): analogue inputs from analysis system transducers, (b): analogue inputs from calorimeter transducers, (c): analogue inputs from calibration system, (d): analogue input / output lines to gas selector switch valves, (e): digital input / output lines to calibration system

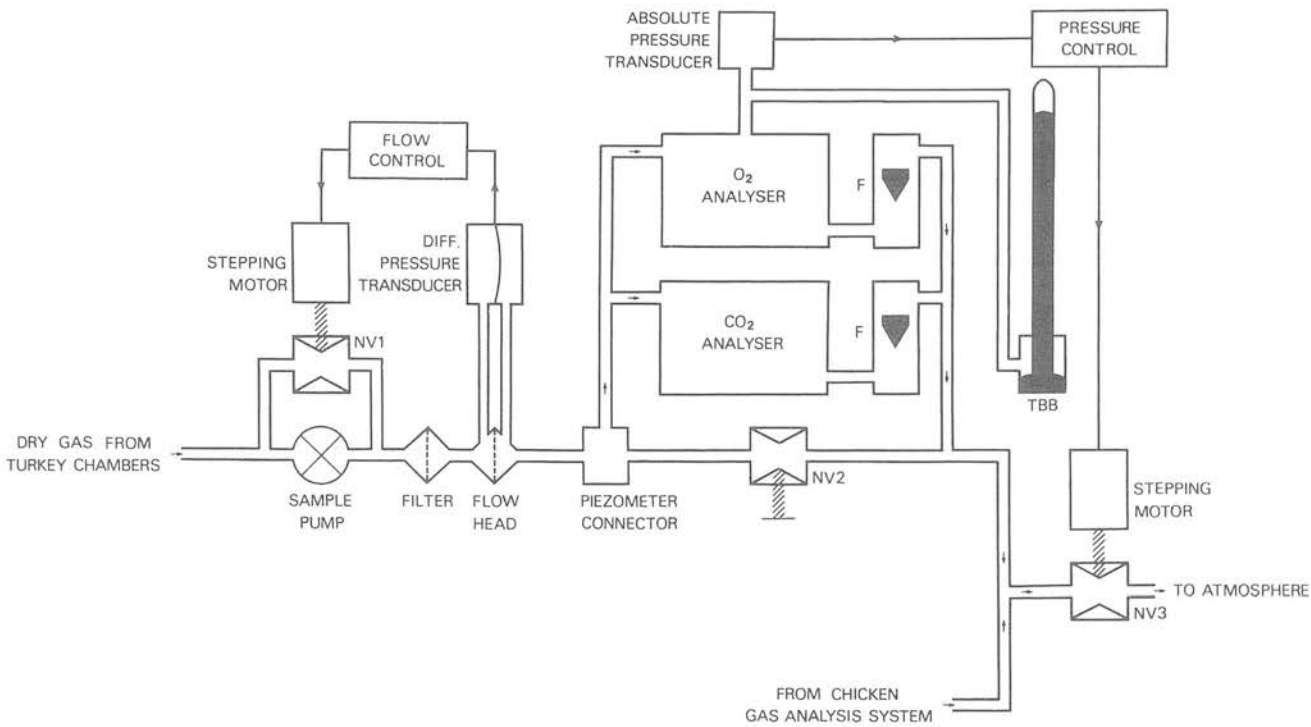


Figure 2.4 *The controlled pressure and flow analysis system (MacLeod et al., 1985)*

F float-and-cone flowmeter

NV needle-valve

TBB test-bench barometer

2.2.2. Heat production calculation

The O_2 consumption and CO_2 production of the birds are calculated in the conventional manner from the difference between the volumes of these gases entering and leaving the calorimetry chamber during the period of observation (10 min). In order to remove 'lag' effects, a correction is made for the change in the O_2 and CO_2 content of the calorimeter during this period. The heat production (H, kJ) was calculated using the Brouwer equation (Brouwer, 1957), modified for poultry by Romijn and Lockhorst (1961). The formula of Romijn and Lockhorst (1961), which is converted to SI units, from O_2 consumption and CO_2 production (dm^3 STP): $H = 16.20\ O_2 + 5.00\ CO_2$ is used to calculate a heat production. No correction is made for nitrogen excretion, since Romijn and Lockhorst (1961, 1966) pointed that the error resulting from this omission is about 0.2% and did not exceed 1.5% even at a high rate of protein catabolism.

2.3. Feed formulation

A linear programming package (FORMAT) was used for experimental diet formulation. The lowest diet and the highest diet were formulated with the program and intermediate diets were calculated by interpolation.

2.4. Excreta collection

Excreta were collected in polymethacrylate (Perspex) trays located on the bottom of the chamber. Daily collections were made over 3 d during feeding and over the

second day of fasting. Collected samples were stored at –20 °C in sealed aluminium dishes until they were freeze-dried and ground for nitrogen and energy analysis.

2.5. Nitrogen analysis

Nitrogen content (g/kg) of diet and excreta were measured by LECO FP328 nitrogen anlayser which measures nitrogen in the gaseous phase after combustion of the sample (based on the Dumas technique).

2.6. Energy analysis

Energy content (kJ/g) of diet and excreta were measured by isoperibol bomb calorimeter (PARR 1261).

2.7. Energy calculation

Energy and protein metabolisability values were calculated by the methods of Sibbald (1976) and McNab and Blair (1988).

True metabolisable energy (TME) was calculated as

TME intake (I_{TME}) = I_E – (faecal+urinary)energy + (endogeneous (faecal+urinary) energy)

where I_E is gross energy intake. Endogeneous energy was measured by analysing the excretion after 24 h fasting.

For the calculation of energy retention and partition the total energy retention (R_E) was as :

$R_E = I_E$ –((faecal + urinary) energy) – H

And N retention (R_N) was calculated as

$R_N = I_N$ – ((faecal + urinary) N)

where R_N is nitrogen retained in the body and I_N is N intake in the diet .

Crude protein (CP) retention could then be calculated by multipying nitrogen retention in grams by 6.25. Energy retained as CP was given by 23.7 × 6.25 R_N. Energy retained as fat could be calculated by substracting energy retained as CP from R_E.

2.8. Statistical analysis

Statistical analysis of the experiments were carried out using GenStat. Detailed methods were given in each experimental chapters.

Chapter 3

EFFECT OF CONSTANT LYSINE AND VARYING CRUDE PROTEIN

3.1. Introduction

As mentioned in Chapter 1, dietary protein quality influences growth but also affects the environment because of different rates of nitrogen excretion. A reduction in nitrogen excretion and increased efficiency of nitrogen deposition can be achieved by matching the amino acid composition of the diet with amino acid requirements. This would also be expected to have implications for energy metabolism. The conventional expectation has been that a balanced blend of amino acids must lead to decreased energetic costs of catabolism and excretion (Baldini, 1961, Guillaume and Summers, 1970). However, MacLeod (1997) reported that heat production on an imbalanced, lysine-limited, amino acid mixture was no higher than on a balanced amino acid source with the same lysine concentration and there was no indication of stimulation of heat production by excess amino acids. Boorman and Ellis (1996) found no adverse effect of protein quality on lysine utilisation and detected some indication of increasing net utilisation with decreased protein quality.

However, the experiment of MacLeod (1997) used a rather heterogeneous series of diets and it was felt that a more linear series of diets would lead to more secure conclusions. This experiment was, therefore, based on diets formulated to have a wide range of linearly varying protein concentrations but equal concentrations of lysine, the first-limiting amino acid. The hypothesis being tested was that growth

rate would be fixed by lysine concentration and that the energy cost of nitrogen excretion would therefore vary with excess nitrogen intake.

The effect of diets with increasing concentration of crude protein at fixed essential amino acid concentrations on performance was also examined. Some studies have indicated that the requirement for the essential amino acids is a function of the total crude protein content of the diet if maximum growth rate is required (*e.g.* Grau, 1948; Almquist, 1952; Morris *et al.*, 1987; Abebe and Morris, 1990). Morris *et al.* (1999) discussed 6 alternative explanations for the limited response to the critical amino acids; 1) limiting energy supply, 2) heat disposal was limiting, 3) growth rate was used as a criterion rather than protein accretion, 4) availability of the amino acids was lower than assumed, 5) nutrients other than amino acids were limiting and 6) an imbalance of amino acids in the diets. They concluded that the most likely explanation was that the limited response stems from a general imbalance of amino acids at high CP intakes. They reported that an amino acid imbalance leads to the decline in the efficiency of utilisation of the limiting amino acid at high CP intake where all amino acids except one are supplied at amounts exceeding the requirements. However, many reports (*e.g.* D'Mello, 1990; MacLeod, 1997) demonstrated that the utilization of the first limiting amino acid was not affected by amino acid imbalance. Although there have been many hypotheses, no complete explanation has been provided so far.

The detailed relationships among amino acid balance, nitrogen metabolism and energy metabolism were investigated in a computer-controlled chamber calorimetry system. Effects of nitrogen metabolism on water intake were of practical importance and were also quantified. The experimental results were compared with simulations

using the mechanistically based model of energy metabolism described by MacLeod (See section 1.2.3.1 and Figure 1.2).

3.2. Materials and methods

3.2.1. Experimental animals

The experiment was performed with growing broiler chickens (21 d old) to examine five diets. Male broiler chicks (1 d old) from the same commercial line (Ross) were obtained in batches at 14 d intervals (so that they were of identical age in each period) and reared to 20 d of age on a common diet in a brooder. At 21 d of age, chicks were randomly allocated (in pairs) to calorimeter chambers, where they remained for 14 d for the experiment.

3.2.2. Experimental design

The experiment was designed as a Latin Square (5 diets × 5 calorimeter chambers) with five 2-week measurement periods; there were therefore 5 replicate pairs of birds on each diet, giving a total of 50 birds used in the experiment.

3.2.3. Diets and environmental conditions

The lysine concentration of each experimental diet was a constant 11 g/kg with CP concentration ranging from 180 g/kg to 300 g/kg (Table 3.1). The experiment had an acclimatization period of 7 days in which animals were allowed to become

accustomed to the experimental diet and calorimeter chamber. The five replicate pairs of birds on each diet were allocated to five two-week periods and five treatments. Calorimeter chambers were kept at 20 °C and on a lighting cycle of 23 h light: 1 h dark which is commonly used in commercial broiler husbandry. The 1h of dark period started at 00.00 and ended at 01.00h. The experimental diet and water were given *ad libitum.*

3.2.4. Measurements and sample collections

Food intake, water intake, body-weight gain and heat production were measured daily. Heat production (HP) was measured by means of the indirect calorimetry apparatus described in chapter 2. The measurements were made from d 28 to d 32. After the first 3 d of measurement, the birds were fasted for 24 h to allow them to reach a basal level of metabolism. Fasting HP and endogenous faecal and urinary energy and nitrogen losses were then measured during a second 24 h period of fasting.

Daily droppings collections were made over 3 d during feeding and over the second day of fasting. Collected samples were stored at –20 °C in sealed aluminium dishes until they were freeze-dried, weighed and ground for nitrogen and energy analysis. Each diet and droppings sample was measured in duplicate and the mean was calculated. Results were expressed per bird per day. The calculations of energy and nitrogen contents and the apparatus used for measuring them in the diet or droppings samples are described in Section 2.4, 2.5 and 2.6.

Table 3.1 *Specifications and ingredients of diets in calorimetry experiment 1*

Diet	1	2	3	4	5
TME [1] MJ/kg	13.4	13.4	13.4	13.4	13.4
CP[2] g/kg	180	210	240	270	300
Lysine concentration g/kg	11.0	11.0	11.0	11.0	11.0
Lysine : CP ratio	0.061	0.052	0.046	0.041	0.037
CP:TME ratio	1.34	1.57	1.79	2.01	2.24
Ingredients (g/kg)					
Wheat meal	586.0	552.5	519.0	485.5	452.0
Barley	150.0	112.5	75.0	37.5	0
Maize meal	81.0	60.8	40.5	20.3	0
Maize gluten meal (60% CP)	0	54.5	109.0	163.5	218.0
Soya bean meal (48% CP)	0	61.0	122.0	183.0	244.0
Casein	101.0	75.1	50.1	25.0	0
Choline chloride	0.3	0.3	0.3	0.3	0.3
Fat blend	28.0	31.0	34.1	37.1	40.1
Dicalcium phosphate	11.5	12.1	12.6	13.2	13.7
Sodium chloride	3.8	3.7	3.6	3.5	3.4
Limestone flour	14.7	14.4	14.1	13.8	13.5
Vitamin/mineral supplement	5.0	5.0	5.0	5.0	5.0
Pellet binder	10.0	10.0	10.0	10.0	10.0
Lysine	0.8	0.6	0.4	0.2	0
Arginine	4.70	3.50	2.35	1.18	0
Methionine	2.70	2.03	1.35	0.68	0
Threonine	0.50	0.38	0.25	0.13	0

[1] TME : true metabolisable energy, [2] CP : crude protein

3.2.5. Simulation

The experimental results were compared with simulations using the mechanistically based model of energy metabolism described by MacLeod (1994, 1998; see Section 1.2.3.1). The simulations were done using the mean bird weight at time of measurement and the mean food intake.

3.2.5. Statistical analysis

Statistical analysis was by analysis of variance, fitting the effects of diet, column (chamber) and row (measurement period) in the model.

$$X = \text{Mean} + \text{Diet} + \text{Chamber} + \text{Period} + \text{Residual}$$

Least-squares regression was also performed using GenStat.

3.3. Results

3.3.1. Food intake, water intake, growth and N utilisation

The results are summarised in Tables 3.2 and 3.3. There was no significant effect of varying CP concentration on food intake. However, CP concentration had a significant effect on the water intake ($P<0.01$). Water intake increased as CP concentration increased. It was about 25% greater with birds on the high protein diet than on the moderate protein diet. Dry matter excretion was also significantly affected by CP concentration ($P=0.001$). No significant difference was found between the birds in body-weight gain. However, the birds fed on the 18% CP diet showed 25% higher body-weight gain compared with the birds fed on the 30% CP diet. Food conversion efficiency (FCE) was not affected by CP concentration. Although the birds fed on the highest protein diet showed slightly lower FCE, there was no significant difference between treatments.

Since there was no significant difference in food intake, there was a significant increase in N intake as CP concentration increased; the increase was by about 52% between the lowest and highest CP diets (Figure 3.1.a). However, N retention did not change significantly as diet protein content increased. N loss in excreta significantly increased with CP content ($P<0.001$). CP content had highly significant effects on efficiency of N utilisation ($P<0.001$). The birds fed on the highest CP diet had the lowest efficiency of N utilisation, resulting in a increase in N excretion of about 150% compared with birds fed on moderate CP diet. The efficiency of N utilisation was maximal in Diet 1 and significantly decreased as CP concentration increased (Figure 3.1.b).

Table 3.2 *Effect of crude protein(CP) content on food intake, water intake, weight gain, food conversion efficiency (FCE) and dry matter(DM) excretion*

Diet	1	2	3	4	5	SED	*P*
CP (%)	18	21	24	27	30		
Food intake (g / bird. d)	132.1	127.3	126.6	123.7	129.1	4.99	NS
Food intake (g / $kgW^{0.75}$.d)	119.8	113.4	112.7	111.6	117.0	3.99	NS
Water intake (ml / bird. d)	170.5	172.8	181.8	195.0	214.7	11.65	0.016
Water intake (ml / $kgW^{0.75}$ .d)	154.7	153.6	161.8	175.7	194.7	10.03	0.008
Water : Food ratio	1.29	1.35	1.44	1.57	1.66	0.05	<0.001
Weight gain (g / bird. d)	80.0	74.0	76.9	75.0	67.7	6.62	NS
FCE	0.61	0.58	0.61	0.60	0.53	0.04	NS
Excretion, DM (g / bird. d)	30.7	32.8	34.9	39.0	41.6	2.11	0.002
Excretion, DM (g / $kgW^{0.75}$.d)	27.9	29.2	31.1	35.2	37.7	1.84	0.001

Table 3.3 *Effect of crude protein(CP) content on nitrogen(N) intake, retention and loss, and efficiency of nitrogen utilization*

Diet	1	2	3	4	5	SED	*P*
CP (%)	18	21	24	27	30		
N intake (g / bird.d)	4.10	4.18	5.29	5.90	6.18	0.21	<0.001
N intake (g / $kgW^{0.75}$.d)	3.70	3.72	4.72	5.32	5.61	0.18	<0.001
N retention (g / bird.d)	2.68	2.43	2.60	2.61	2.60	0.15	NS
N retention (g / $kgW^{0.75}$.d)	2.44	2.16	2.32	2.36	2.35	0.12	NS
N loss (g / bird.d)	1.41	1.75	2.68	3.29	3.59	0.17	<0.001
N loss (g / $kgW^{0.75}$.d)	1.28	1.56	2.40	2.97	3.26	0.15	<0.001
Efficiency of N retention	0.66	0.58	0.49	0.44	0.42	0.022	<0.001

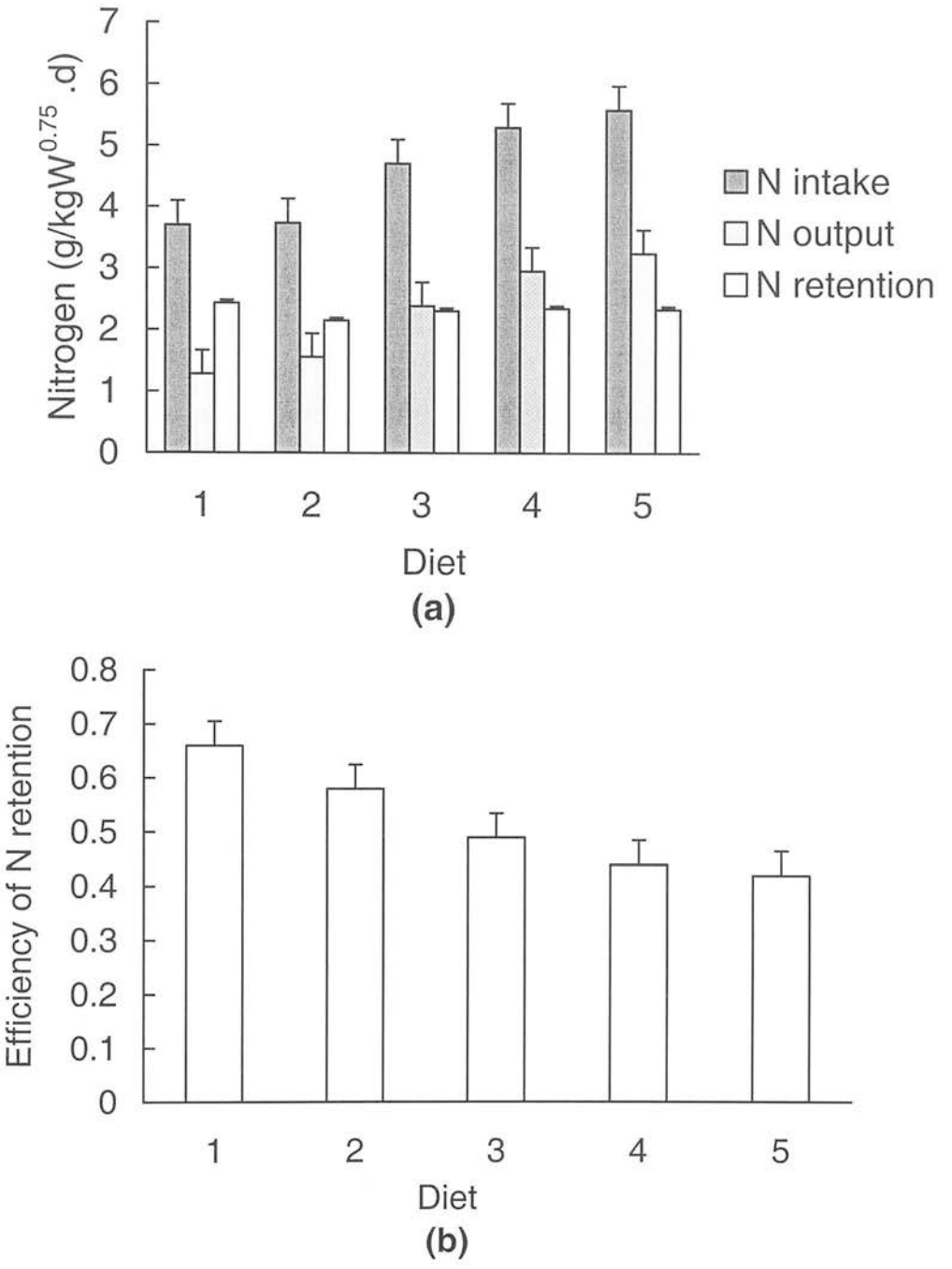


Figure 3.1 *Effect of crude protein(CP) content on nitrogen(N) utilisation*

(a) *Effect of CP content on nitrogen intake, loss, and retention*

(b) *Effect of CP content on efficiency of nitrogen retention*

** CP content of diets : 1, 18 %; 2, 21%; 3, 24%; 4, 27%; 5, 30%. Standard error bars were calculated from the pooled estimate of the results.*

3.3.2. Heat production and energy utilisation

Table 3.4 and Figure 3.2 show the results for heat production and energy utilisation. There was no significant effect of amino acid balance on heat production despite the very large change in N excretion. Simulated heat production agreed with the empirically determined results in not showing a trend with dietary CP.

True metabolisable energy (ME corrected for endogenous energy losses, see Section 2.6) intake values are shown in Table 3.4. Although no significant difference in TME intake was detected between diets, there was a tendency for TME intake to be lower on higher CP diets. TME intakes were higher on the moderate protein, balanced diets (Diet 1 and 2) than high protein, imbalanced diets (Diet 3, 4 and 5).

Protein, fat and total energy retentions are presented in Table 3.4. Total energy retention differ significantly ($P<0.05$) between varying CP diets. Energy retention as protein was similar between diets. Energy retention as fat between diets were significantly different ($P=0.001$) and there was a tendency to be lower on higher CP diets.

Table 3.4 *Effect of crude protein(CP) content on heat production(HP) and energy(E) utilisation*

Diet	1	2	3	4	5	SED	*P*
CP (%)	18	21	24	27	30		
TME intake (kJ / bird. d)	1870	1918	1824	1844	1763	64.5	NS
TME intake (kJ / $kgW^{0.75}$.d)	1696	1708	1622	1663	1598	48.3	NS
Heat production (kJ / bird. d)	1037	1021	1038	988	999	33.4	NS
Heat production (kJ / $kgW^{0.75}$.d)	939.7	908.9	928.3	890.7	902.3	26.24	NS
Simulated HP (kJ/bird.d) *	977	977	970	978	979	-	-
E retention (kJ / bird. d)	725	800	680	754	654	45.7	0.056
E retention (kJ / $kgW^{0.75}$.d)	657	712	604	680	593	37.2	0.039
CP retention (g / $kgW^{0.75}$.d)	16.8	15.8	16.3	16.3	16.2	0.92	NS
E retention as protein (kJ/bird.d)	398	360	386	387	384	21.76	NS
E ret as protein (kJ / $kgW^{0.75}$.d)	361	320	343	349	348	17.13	NS
E retention as fat (kJ / bird. d)	328	440	294	367	270	30.84	0.001
E retention as fat (kJ / $kgW^{0.75}$.d)	297	393	261	331	245	26.68	0.001

* The simulation results cannot be given a standard error since the model is not stochastic.

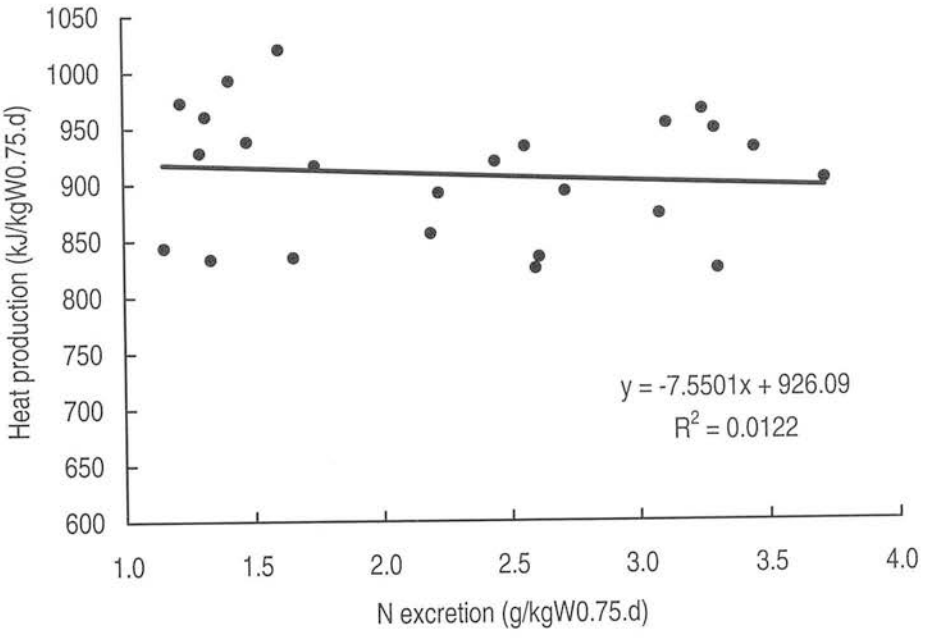


Figure 3.2 *The relationship between heat production (HP) and nitrogen (N) excretion. Standard errors are shown in square brackets. HP = -7.55[14.5]N_{Out} + 926.1[35.2], (P = 0.608)*

3.4. Discussion

3.4.1. Food intake, water intake and growth

The results indicated that there was no effect of CP concentration ranging from 18 to 30 % of diets on food intake. It seems that control of energy intake or first limiting amino acid took priority over the control of CP intake. MacLeod (1990) reported that there was no effect of CP concentration on food intake when he fed diets ranging from 130 to 260 g/kg. These results are also in agreement with Shariatmadari and Forbes (1993) who reported that there was no significant difference in food intake and growth rate between diets ranging from 172 to 280 CP g/kg. Morris *et al.* (1987) examined the effect of diets varying in CP content (140 to 280 g/kg diet) on male broiler chicks. They reported that growth rate and efficiency of food utilisation to 21 d of age responded to increasing dietary protein contents up to about 230 g/kg CP. According to Gous and Morris (1985) and Abebe and Morris (1990), the concentration of the first limiting amino acid had the most important effect on growth and food intake. The results from the present experiment confirm that growth rates were fixed by the concentration of the first limiting amino acid. Although the food intake of birds on different diets did not differ significantly, those on the lowest protein diet (18% CP) tended to have a slightly higher food intake than other groups. It has been suggested that with a diet containing slightly less protein than optimal, food intake might increase modestly over that for an optimal diet (Boorman, 1979) as the birds try to attain a protein intake commensurate with their needs.

When protein content is much below the required level or the ratio of protein to energy was decreased, the food consumption decreases (Gleaves *et al.*, 1968). They

reported that food intake was reduced when the CP:ME ratio was decreased and *vice-versa*. They attributed the greater food intake when the ratio increased to the increase in egg production, where egg production was associated with protein level. Protein concentration, therefore, acted on food intake in an indirect way. They also suggested that energy in the diet should be adequate, otherwise protein would be used as a source of energy but, when energy is beyond the requirement level, it will serve as the main regulator of food intake. Fancher and Jensen (1989) fed male broilers diets varying CP levels below 20% and reported that the minimum CP level needed to optimize body weight ranged from 16 to 17.5% whereas feed efficiency required that the CP be greater than 18.9%.

Although differences were not significant, there was a tendency for growth to be lower on higher CP diets in the present experiment. Sklan and Plavnik (2002) reported that increasing protein content from 182 to 242 g/kg resulted in a linear decrease in food intake, while weight gain and feed efficiency changed quadratically with a smaller positive effect at the highest crude protein intakes. They suggested that broiler performance at the lower protein intakes was limited by either non-essential amino acid or essential amino acid intake whereas at high protein intakes the decreased efficiency of amino acid utilisation after growth requirements are fulfilled resulted in poorer performance.

Water intake was significantly affected by the CP content of the diet. Water: feed ratio also increased from 1.29 up to 1.66 as CP content increased from 18 to 30% (Table 3.2). Marks and Pesti (1984) reported that increasing CP content increased the water intake and water: feed ratios. They found that birds receiving a 26% protein diet consumed more water than birds receiving the 22% and 17% diets (124,

108 and 86 g/d from d 16-18 respectively) and concluded that water: feed ratios were directly proportional to dietary protein content. James and Wheeler (1949) and Wheeler and James (1950) firstly showed that the amount of water consumed as well as the amount of droppings produced varied almost directly with the percent of protein in the diet. Patrick and Ferrise (1961) stated that greater amounts of water are required for the metabolism of protein than for carbohydrates or fat. A partial explanation for this relationship may be that more water is needed to increase the elimination of nitrogen as uric acid. Since the bird is incapable of breaking down uric acid into urea, an appreciable amount of water consumed is, therefore, tied up in the metabolism of the excess of protein ingested.

3.4.2. Nitrogen utilisation

It is generally recognised that reducing the dietary protein concentration is the most efficient way to lower nitrogen excretion. According to Summers (1993), when dietary CP was lowered 17 to 13% for 24-wk-old layers, faecal N excretion was reduced as much as 34% without affecting egg production. Several researchers (Moran and Bushong, 1992; Moran, 1994) have shown that reducing the dietary protein content to 10 and 15% while maintaining the required essential amino acid levels within each age period for broilers, will reduce litter N content approximately 24% without impairing weight gain. In this experiment, increasing dietary CP level by 67% and nitrogen intake by 1.91 g/d resulted in a 155% (1.98 g/d) increase in nitrogen excretion (Figure 3.3). Ferguson *et al.* (1998) reported that each 1% reduction in the protein level of the broiler diet resulted in a 7% reduction in N output. Blair *et al.* (1999) showed that reduction in dietary CP content caused a 10-

27% reduction in the total amount of nitrogen excreted during the 6-week broiler rearing period and with layers, there was a 30-35% reduction in daily N output. They also reported that reduction in dietary CP from about 21 to 18% resulted in a reduction of more than 20% in daily N output. This is similar to the result in present experiment which showed 20% reduction in N output as CP level decreased from 21 to 18%. The efficiency of N utilisation decreased as CP content increased. Overall, reduction of dietary CP level had a beneficial effect in terms of reducing N excretion.

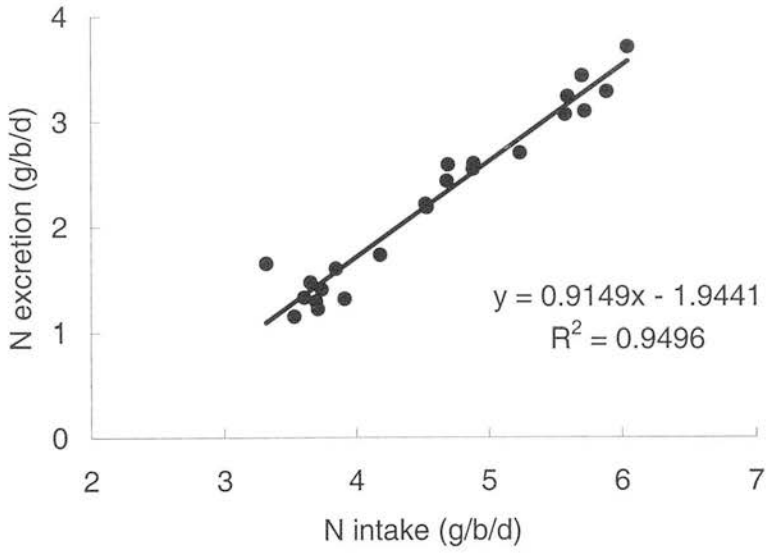


Figure 3.3 *Linear regression of nitrogen excretion (Y) on nitrogen intake (X) of birds*

3.4.3. Heat production and energy utilisation

Theoretically animals fed protein in excess of their requirements must catabolise the surplus amino acids, leading to increased excretion of nitrogen, and this process yields heat. Both the synthesis and degradation of protein and amino acids are energy demanding processes and these costs are necessarily included in model predictions of energy balance. Also, when protein synthesis becomes limited by the first limiting amino acid, amino acids present in excess of the resulting requirement enter the pool of substrates available as energy sources. It has been implied that this would result in a greater heat increment than would occur with a balanced amino acid mixture. In birds, the excretion of excess amino acid N which is not used for protein synthesis takes place mainly as uric acid. Uric acid synthesis is approximately twice as energy demanding as urea synthesis, and so birds inherit an energy cost for excreting an insoluble nitrogenous product. However, uric acid has four of N atoms and urea has two N. Therefore, the total energy cost is about the same per each of N atom excreted. According to MacLeod (1998), the trans-species N correction of 5.63 kJ/g of protein retained (35.2 kJ/g N) agrees closely with the 36.5 kJ/g N conventionally used in poultry.

As described in the introduction (see Section 1.2.3.1), Emmans (1994) reported the method to predict the 'effective energy' of diets. He suggested that heat increment of feeding is linearly related to five measurable traits; faecal organic matter, urinary nitrogen (UN), nitrogen retention, lipid retention derived from dietary lipid and fat retention derived from non-lipid feed ingredients. The estimates for theses process of the heat increment are 3.81, 29.20, 36.48, 16.40 and 4.39 kJ/g product, respectively.

According to his calculation, 29.2 kJ of energy cost is needed for the excretion of g of UN.

Digestibility of protein varies with ingredients of the diet. But typical digestibility of nitrogen in the diet is approximately 75% which means about 25% of nitrogen intake will be lost in the faeces. Therefore, the amount of urinary N can be estimated from the total N loss in the faeces. In the present experiment, therefore, the heat increment of UN excretion of each treatment can be calculated as 9.93, 18.4, 35.6, 47.9 and 54.3 kJ / bird. d, respectively. HP of each group ranged between 890 and 940 $kJ/kgW^{0.75}.d$ and no significant difference was detected. The estimated values of energy cost of N excretion are about 1 to 6% of total HP which is relatively small. The largest part of heat increment of feeding is protein retention which costs 36.48 kJ/g. N retention of five treatments was constant in the present experiment. Energy cost of protein retention, therefore, seems to have not affected the total HP.

Although there was fluctuation in fat retention, there was a tendency for it to decrease as protein content increased. The decrease in energy retained as fat, as CP level increases in the diets, was reported by Adams *et al.* (1962a) and MacLeod (1990, 1992). The reduction in protein concentration means that a greater proportion of energy will be available from the diet to fuel the different processes which require energy. This, in turn, allows the animal to store a higher proportion of its energy intake as body fat. Bartov (1979) stated that excess dietary protein also forced the birds to use energy to excrete excess nitrogen as uric acid. Therefore, less energy would be available for lipogenesis (Buttery and Boorman, 1976).

3.5. Conclusion

The experiment was performed to test the hypothesis that growth would be determined by lysine concentration and that the energy cost of nitrogen excretion would vary with excess nitrogen intake. The results showed that there was no significant effect of varying CP content on food intake and growth. This suggests that growth rates were fixed by the concentration of the first limiting amino acid or dietary energy content. Since the ME content of the diet and food intake were within normal limits, energy intake is unlikely to have been limiting. However, N loss in excreta significantly increased with increasing CP content. The efficiency of nitrogen utilisation significantly decreased as CP content increased. This confirmed the beneficial effect of reduced CP diet in terms of reducing N excretion.

It was found that total HP was not affected by increasing CP content. The estimated energy cost of N excretion was relatively small and there was no indication of a stimulation of HP by excess amino acids. These results suggest that HP was correlated with the first limiting amino acid intake rather than total CP intake. Further experiments should be planned to investigate this relationship between HP and the first limiting amino acid.

Chapter 4

EFFECT OF CONSTANT CRUDE PROTEIN AND VARYING LYSINE ON HEAT PRODUCTION

4.1. Introduction

In the previous experiment, there was no significant difference in heat production with varying range of protein contents but constant lysine concentration. This result agrees with MacLeod (1997) who found that heat production was closely correlated with the rate of protein accretion (which in turn was strongly associated with the intake of the first limiting amino acid) and was affected little by total crude protein intake. The experiment in this chapter, therefore, was designed to examine the response to diets which contain different concentrations of lysine, as the first-limiting amino acid, at constant high CP content. Some former studies have suggested that when protein synthesis becomes limited by the first limiting amino acid, it would result in a greater heat production. The hypothesis being tested in this experiment was that heat production would vary with growth rate (specifically protein accretion rate), which would itself be controlled by dietary lysine concentration. The detailed relationships among amino acid balance, nitrogen metabolism and energy metabolism were investigated in a computer-controlled chamber calorimetry system as the previous experiment.

4.2. Materials and Methods

Five diets were formulated with lysine concentration ranging from 6 to 14 g/kg, at equal intervals of 2 g/kg (Table 4.1). This range was achieved by supplementing a basal diet with 0 to 10 g/kg of L-lysine HCl (Table 4.2). The diets were kept isonitrogenous by substitution of glutamic acid as lysine concentration increased (Table 4.3). Other indispensable amino acids were maintained at concentrations 1.5 times the published recommendation for growing broilers. Detailed procedure and measurements were same as previous experiment described in the previous chapter (see Section 3.2).

Table 4.1 *Specifications of experimental diets in calorimetry experiment 2*

Diet	6	7	8	9	10
True metabolisable energy (TME) MJ/kg	13.4	13.4	13.4	13.4	13.4
Crude protein (CP) g/kg	300	300	300	300	300
Lysine concentration g/kg	6.0	8.0	10.0	12.0	14.0
Lysine : CP ratio	0.020	0.027	0.033	0.040	0.047

Table 4.2 *Formulation of basal diets in calorimetry experiment 2*

Diet	g/kg
Wheat meal	468.8
Barley	86.8
Maize gluten meal (60% CP)	346.5
Maize oil	10
Choline chloride	0.3
Fish meal	9.6
Dicalcium phosphate	29.1
Sodium chloride	2.9
Limestone flour	15
Vitamin/mineral supplement	5
Pellet binder	10
Arginine	8
Methionine	1.4
Threonine	1.83
Tryptophan	0.8

Table 4.3 *Composition of supplements in calorimetry experiment 2*

Diet	Supplements (g/kg of diet)		
	Lysine	Glutamic acid	Maize starch
6	0	15.83	0
7	2.5	11.88	1.45
8	5.0	7.92	2.91
9	7.5	3.95	4.38
10	10.0	0	5.83

4.3 Results

4.3.1. Food intake, water intake and growth

Table 4.4 shows the results of intake, growth and excretion. Food intake increased as lysine concentration increased ($P<0.05$). The birds fed on diet 9 (1.2% lysine) showed the highest food intake and consumed 20% more food when compared with birds fed on diet 6 (0.6% lysine). Food intake of the birds fed on diet 10 (1.4% lysine) was slightly lower than in the birds fed on diet 9. However, this significant difference disappeared when food intake was adjusted for body-weight.

Lysine concentration also influenced water intake ($P=0.007$). It increased by 100% in the birds fed on diet 9 when compared with the birds fed on the lowest lysine diet. Dry matter excretion differed significantly ($P=0.05$) but not when expressed in terms of $W^{0.75}$.

Daily body-weight gain increased significantly as lysine concentration increased ($P<0.001$). However, Diet 10 (1.4% lysine) resulted in a reduction in body-weight gain compared with Diet 9. There was a 3-fold range in daily weight gain with variation in dietary lysine concentration (Figure 4.1(b)). The groups fed on lysine deficient diets (Diet 6, 7) showed much lower growth rate than the groups fed on the diets which had higher lysine concentration.

Table 4.4 *Effect of lysine concentration on food intake, water intake, weight gain, food conversion efficiency (FCE) and dry matter excretion*

Diet No.	6	7	8	9	10	SED	*P*
Lysine (g/kg)	6.0	8.0	10.0	12.0	14.0		
Food intake (g/bird.d)	88	107	104	133	118	11.9	<0.05
Food intake (g/kgW $^{0.75}$.d)	96	105	100	116	107	8.0	NS
Water intake (ml/bird.d)	130.8	196.2	205.6	262.4	235.6	22.45	<0.001
Water intake (ml/kgW$^{0.75}$.d)	142.6	193.4	197.6	229.8	214.8	19.17	0.007
Water : Food ratio	1.49	1.88	1.95	1.97	2.00	0.17	0.058
Wt gain (g/bird.d)	31.3	53.6	67.4	95.1	85.3	9.06	<0.001
FCE	0.36	0.50	0.65	0.71	0.72	0.06	<0.001
Excretion, DM(g/bird. d)	25.4	29.8	26.8	37.7	32.5	3.79	0.05
Excretion, DM (g/kgW$^{0.75}$.d)	27.8	29.1	26.0	33.0	29.6	2.84	NS

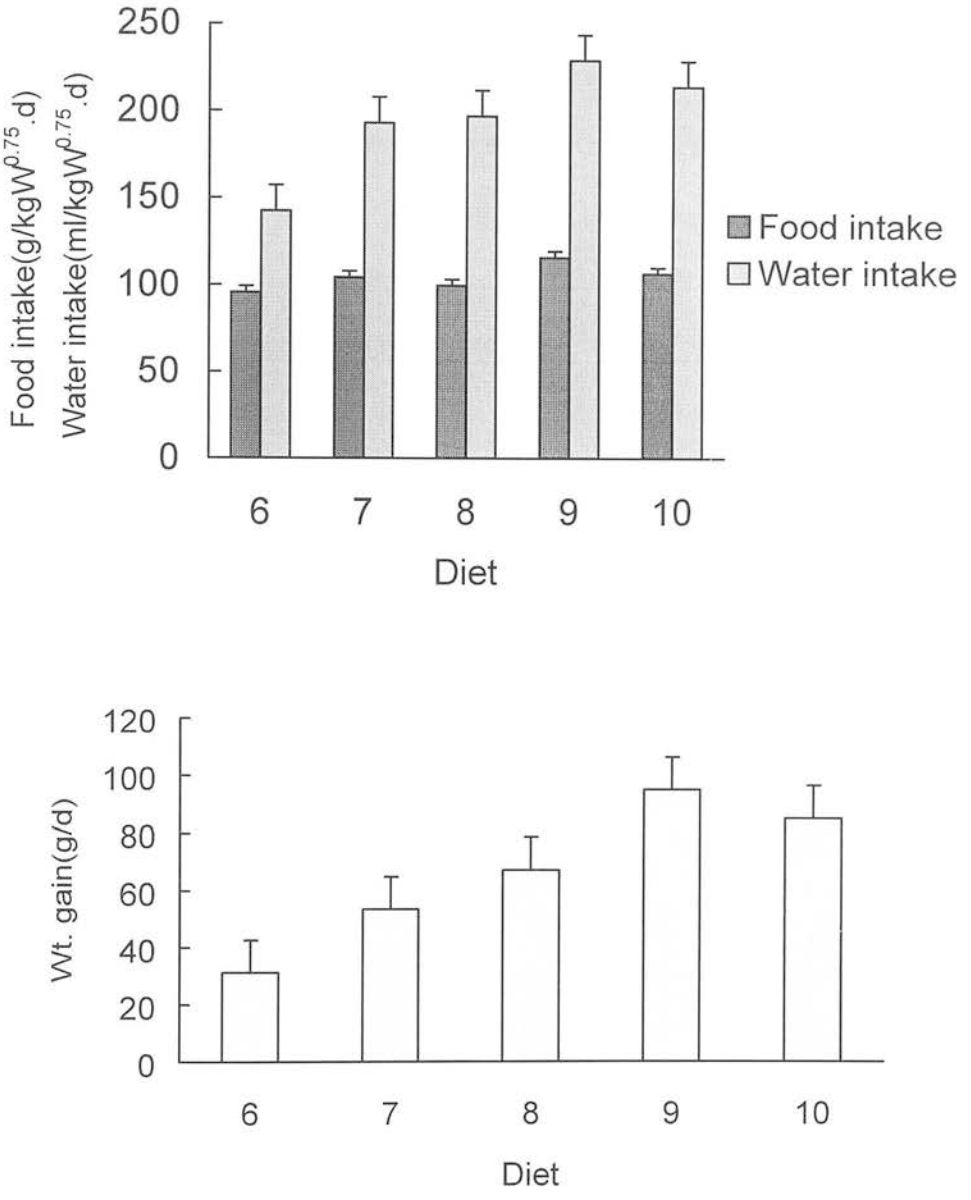


Figure 4.1 *Effect of lysine concentration on intakes and weight gain. Lysine concentration of diets (g/kg) : 6, 6.0; 7, 8.0; 8, 10.0; 9, 12.0; 10, 14.0. Standard error bars were calculated from the pooled estimate of the results.*

4.3.2. Nitrogen utilisation

Table 4.5 shows the effect of lysine concentration on N utilisation. Lysine concentration had a significant effect on N intake (P=0.012). However the significance disappeared when the measurement was adjusted for body-weight. N loss was not significantly different between diets but there was a tendency for it to increase as lysine concentration increased. N retention was significantly affected by lysine concentration and therefore lysine intake (P=0.02). N retention was highest in the birds fed on diet 9 and the increase was by 53% compared with diet 6, the lowest lysine diet. Efficiency of N retention significantly increased as lysine concentration increased (P=0.018, Figure 4.2).

Table 4.5 *Effect of lysine concentration on nitrogen utilisation*

Diet No.	6	7	8	9	10	SED	*P*
Lysine (g/kg)	6.0	8.0	10.0	12.0	14.0		
N intake (g/bird.d)	4.39	5.35	5.14	6.95	5.91	0.597	0.012
N intake (g/kg$W^{0.75}$.d)	4.82	5.23	4.93	6.08	5.38	0.404	0.06
N retention (g/bird.d)	1.68	2.08	2.40	3.20	2.81	0.354	0.008
N retention (g/kg$W^{0.75}$.d)	1.83	2.04	2.27	2.80	2.56	0.262	0.02
N loss (g/bird.d)	2.72	3.27	2.74	3.75	3.09	0.336	0.051
N loss (g/kg$W^{0.75}$.d)	2.98	3.19	2.66	3.29	2.82	0.241	NS
Efficiency of N retention	0.38	0.39	0.46	0.46	0.48	0.029	0.018

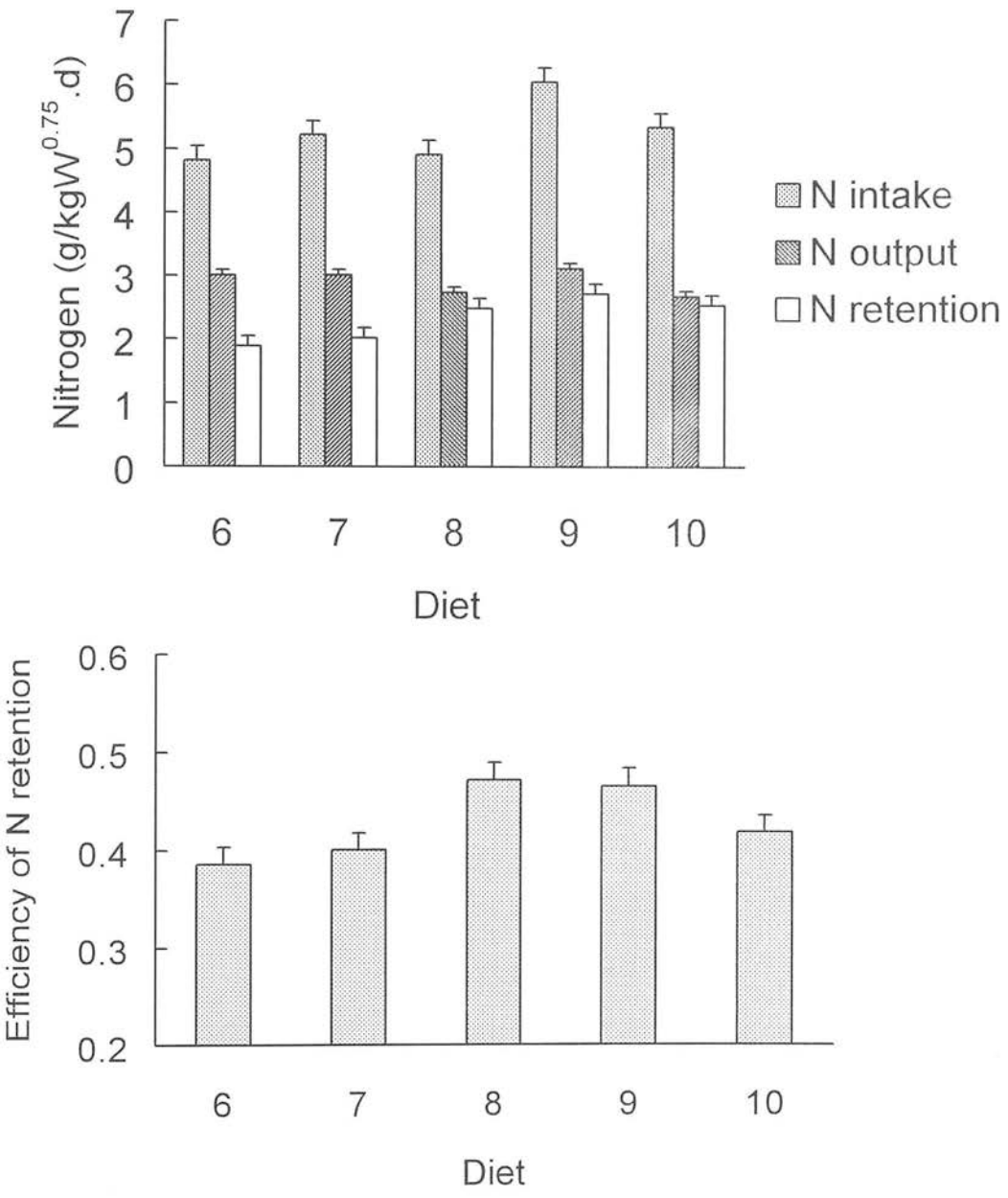


Figure 4.2 *Effect of lysine concentration on nitrogen intake, loss, retention and efficiency of nitrogen retention. Lysine concentration of diets (g/kg) : 6, 6.0; 7, 8.0; 8, 10.0; 9, 12.0; 10, 14.0. Standard error bars were calculated from the pooled estimate of the results.*

4.3.3. Heat production

Heat production per bird increased significantly ($P<0.05$) with rate of growth and therefore with dietary lysine content (Table 4.6). A trend remained when heat production was adjusted for body weight difference ($kgW^{0.75}$) but it was no longer statistically significant. There was a trend for heat production to increase with food intake. Simulated HP results also showed little effect of varying lysine concentration and growth rate on HP.

Table 4.6 *Effect of lysine concentration on heat production and energy utilisation*

Diet	6	7	8	9	10	SED	*P*
Lysine (g/kg)	6.0	8.0	10.0	12.0	14.0		
TME intake (kJ/bird.d)	1174	1431	1394	1780	1576	159.4	<0.05
TME intake (kJ/kgW $^{0.75}$.d)	1287	1400	1338	1558	1435	107.6	NS
E retention (kJ/bird.d)	417	541	508	766	636	108.1	0.06
E retention (kJ/kgW $^{0.75}$.d)	453	532	482	672	578	91.1	NS
Heat production (kJ/bird.d)	757	890	885	1013	940	66.2	<0.05
H P(kJ/kgW $^{0.75}$.d)	832	868	849	890	860	37.6	NS
Simulated HP (kJ/bird.d) *	1049	1002	973	979	985	-	-
CP retention (g / kgW$^{0.75}$.d)	10.5	13.0	15.0	20.0	17.6	2.21	0.008
E retention as protein (kJ/bird.d)	248	308	356	474	417	52.4	0.008
E ret as protein (kJ / kgW$^{0.75}$.d)	271	302	337	414	379	38.8	0.02
E retention as fat (kJ / bird. d)	169	233	153	293	219	69.0	0.324
E retention as fat (kJ/kgW$^{0.75}$.d)	182	230	145	258	198	63.4	0.47

* The simulation results cannot be given a standard error since the model is not stochastic.

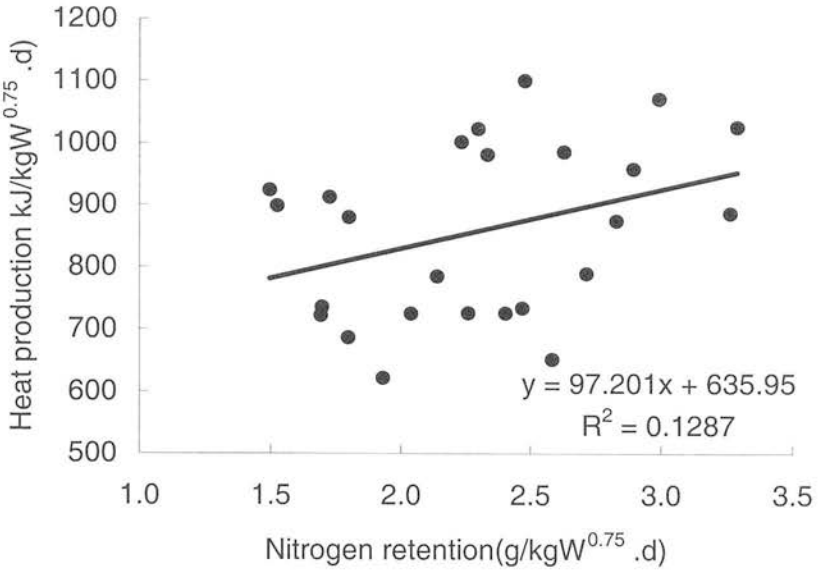


Figure 4.3 *The relationship between nitrogen retention and heat production. Standard errors are shown in square brackets. HP = 97.2[52.7]R_N + 636.0[124.0] (P=0.078)*

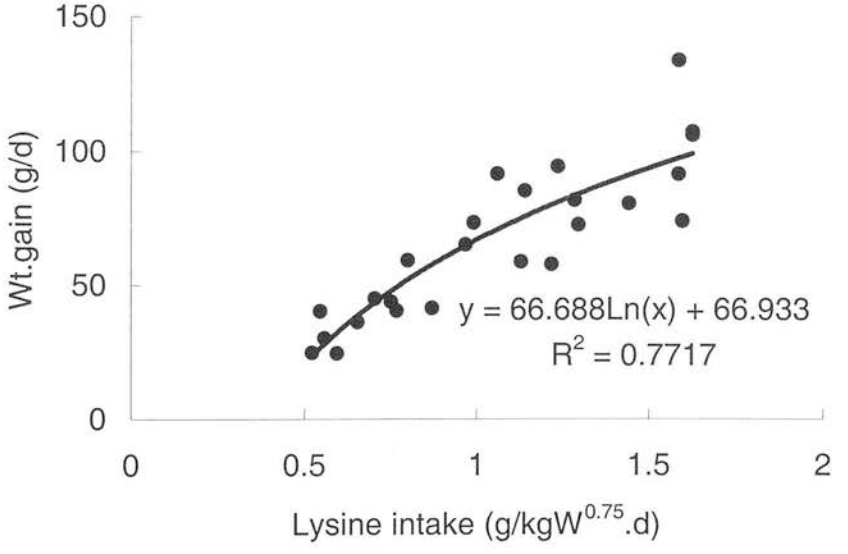


Figure 4.4 *The relationship between lysine intake and body-weight gain*

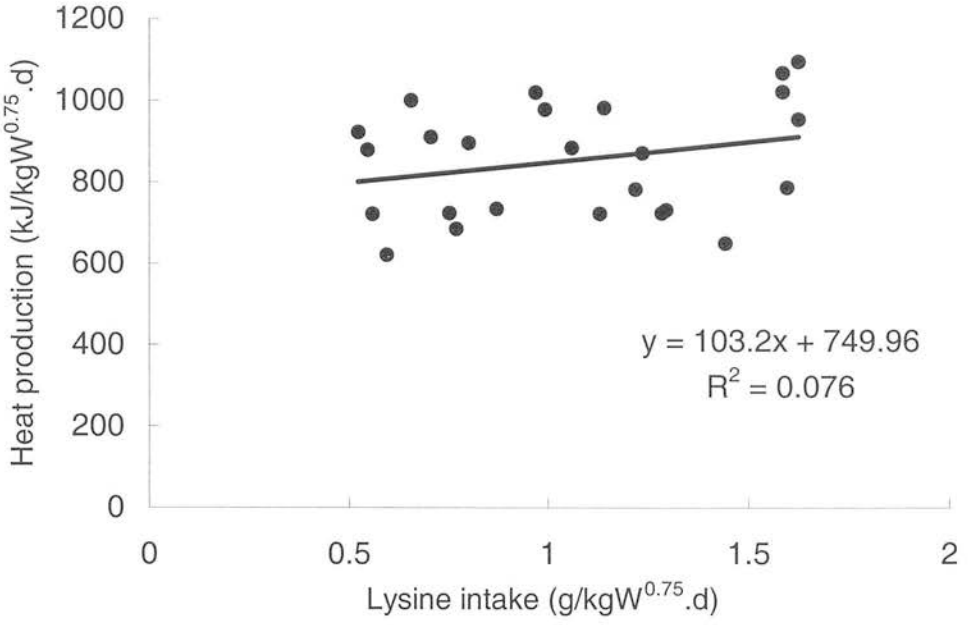


Figure 4.5 *The relationship between lysine intake and heat production. Standard errors are shown in square brackets. HP = 103.2[75.0]I_{Lys} + 750.0[84.3], (P=0.182)*

4.4. Discussion

4.4.1. Food intake, water intake, growth and nitrogen utilisation

There was a significant increase in food intake as lysine concentration increased up to 1.2% of the diet. Diet 6 showed markedly low food intake and growth rate. It appears that there was lysine deficiency due to the low lysine concentration (0.6%) in Diet 6. The food intake decreased slightly in Diet 10 compared with Diet 9. It is known that severe amino acid imbalance primarily results in reduced food intake which, in turn, can affect growth rate *(e.g.* Okumura and Mori, 1979; Summer and Leeson, 1985). Amino acid excess can also result in impaired growth performances (Katz and Baker, 1975; Han and Baker, 1993). Diets which are imbalanced in the amino acids absorbed from the digestive tract lead to metabolic disturbances and a reduction in food intake which is directly proportional to the degree of amino deficiency or imbalance. The effect on intake may be due to the metabolic cost of deaminating the excess of those amino acids which cannot be utilised because of the deficiency, relative or absolute, of others. D'Mello (1994) stated that the abnormal amino acid pattern caused by excess or deficiency of a single amino acid is detected by the appetite regulation system, depresses food intake and reduces growth rate. It is caused by the imbalance in the plasma amino acid affecting appetite directly because of post-ingestional feed back (Figure 4.6). Diet 9 (1.2% lysine) showed the best performance among all treatments. The NRC (1994) recommends that broilers receive 1.0% lysine in the diet (CP 22%) at 3-6 weeks of age. Si *et al.* (2001) examined diets with 4 different levels of lysine (NRC recommendation, +0.1%, +0.2% and +0.3%) in male broiler chickens and reported that there was significant increase in body weight at 21 and 42 d with addition of +0.1% lysine above NRC

recommendation. Kidd and Fancher (2001) also stated that lysine requirement of broiler may vary as meat type broiler genetics are improved. They found that the dietary lysine requirement of the growing broiler is between 107% and 111% of the NRC recommendation (1.18 to 1.22% lysine in the diet).

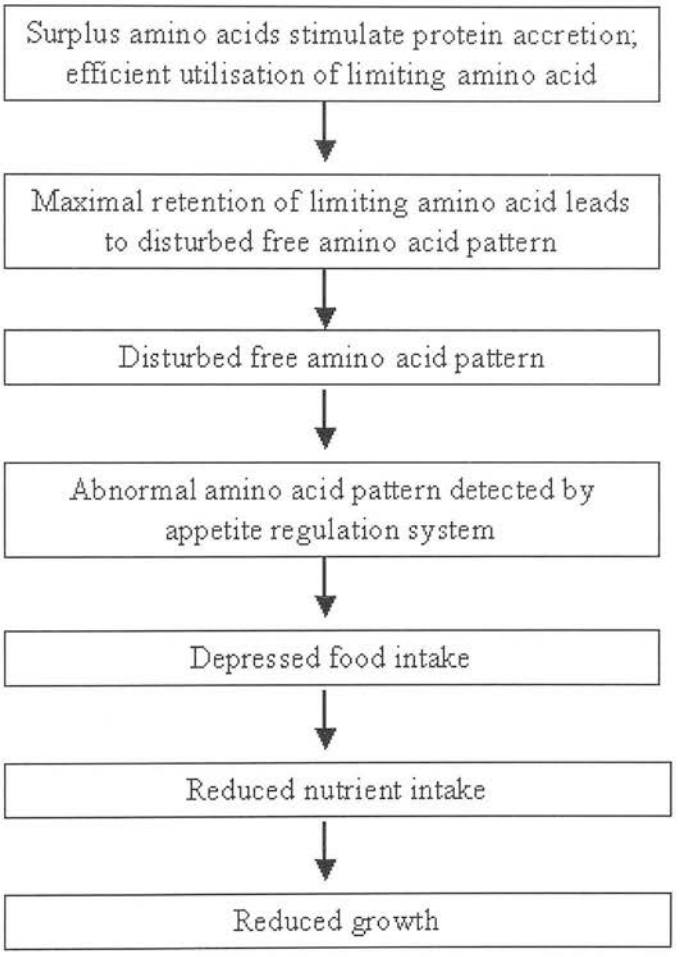


Figure 4.6 *Effects of amino acid imbalance (Adapted from D'Mello (1994))*

It is also probable that the high CP content of diets used in this experiment caused higher lysine requirement. Ever since Grau (1948; cited by Morris *et al.*, 1999) stated that the requirement for an amino acid expressed as a proportion of the diet increases in direct proportion to the protein content, many reports (*e.g.* Mendonca and Jensen, 1989; Barbour *et al.*, 1993; Morris *et al.*, 1987, 1999) have investigated the relationship between dietary protein content and amino acid requirement and they have concluded that the requirement increases as a linear function of dietary protein content. Experimental diets used in the present experiment had 30% CP. Therefore it is possible that the requirement for lysine is higher than the recommendation due to the higher CP content and the birds fed on 1.2% lysine diet showed the best performance. Dietary lysine requirements estimated by previous research vary over a wide range (Lehmann *et al.*, 1996). This variation in results may be due to differences in growth rate and food intake as influenced by genetic and environmental factors such as ambient temperature which affect nutrient utilisation (Withers, 1992).

Water intake increased significantly along with food intake as lysine concentration increased. Generally, water consumption of birds is approximately twice as much as the amount of food consumed although water intake depends on many factors (NRC 1994). In the present experiment, water: feed ratios were between 1.8 and 2.0 except for Diet 6, where it was 1.5.

Although statistical significance decreased when the measurement was adjusted for body-weight, it seems that the first limiting amino acid affected N utilisation. N retention efficiency was significantly improved as lysine concentration increased from 6 to 14 g/kg.

4.4.2. Heat production and energy utilisation

Baldini (1961) reported that heat production was increased by methionine deficient diets. Guillaume and Summers (1970) also found that a diet giving methionine-cystine adequacy with other amino acids fed in relative excess produced an increase in heat production. Although there was some evidence for greater fasting heat production in growing chickens fed on a lysine-deficient diet (Tasaki *et al.*, 1970, 1976) most of the effects on energy utilisation efficiency resulted from a large decrease in food intake. In fact, in most cases where a diet deficient in a single amino acid has been fed, (Okumura and Mori, 1979; Sugahara *et al.*, 1985; Sugahara and Kubo, 1992) the major effects on energy retention have been through a reduction in food intake. Farrell (1976) reported insensitivity of the heat production of previously fasted, tube-fed chickens to whether nitrogen was provided as protein of high quality, protein of low quality, diammonium citrate, or whether they were given a diet containing no nitrogen. In all cases, the heat increment was 0.25 - 0.30, giving a net availability of ME of 0.70 - 0.75. A possible explanation is that all the test diets were used entirely as energy sources because the birds had previously been fasted and that variation due to differences in rates of protein synthesis were therefore not permitted to come into play.

There was a significant relationship between HP and dietary lysine concentration but this was partly due to greater body weight on the higher lysine concentrations and the relationship became less significant when HP was adjusted for body weight. The resultant regression between HP and lysine intake gave less than half the gradient determined by MacLeod (1997), in an experiment using a more diverse range of diets.

This was despite a significant correlation between body weight gain and lysine intake (r=0.77, P<0.001).

The relative energy costs of protein accretion and N excretion can be compared in terms of ATP breakdown and synthesis. The energy cost of incorporating an amino acid molecule into a protein is about 4 mol ATP (Schulz, 1978). Excretion, as uric acid, of the N resulting from catabolism of the amino acid uses 6 mol ATP/g.atom N. Excretion of the N from amino acids therefore costs 6 mol ATP/mol amino acid for most amino acids (which contain 1 N) but as much as 18 for histidine (which contains 3 N). However, the cost of uric acid synthesis is more than offset by the energy resulting from oxidation of the amino acid molecule. The ratio of ATP yield (from oxidation) to ATP utilisation for uric acid synthesis is 68:32 (mean of major dietary amino acids). The positive balance of ATP from oxidised amino acids is potentially available to spare the oxidation of other substrates. Presumably, therefore, the oxidation of excess amino acids need not lead to heat production additional to that resulting from the oxidation of carbohydrates and fats. This was confirmed by the simulation results; where there was a high protein content with low concentrations of the first-limiting amino acid, the simulation programme attributed the energy resulting from oxidation and excretion of the excess amino acids to an increased maintenance heat production. At temperatures below thermoneutral, any additional energy available may also be used for thermoregulation, although the experiment of MacLeod (1997) was also performed at 20 °C and demonstrated a wide range of heat productions in relation to lysine intake and rate of protein accretion. However, to prevent the masking of dietary effects by thermoregulatory demands, further similar experiments are planned at 30 °C. At higher temperatures, it is possible that a further interaction may occur: any additional thermogenic effect

of the diet may lead to reduced food intake if the bird is unable to dissipate additional heat.

4.5. Conclusion

This experiment was planned to investigate the detailed relationship between the first limiting amino acid and energy utilisation. Experimental diets were formulated with varying lysine and constant CP, in contrast to the previous experiment.

The results showed that there was a large difference in growth rate between the lowest and the highest lysine diet. Efficiency of N retention also significantly improved as lysine concentration increased.

HP increased significantly with the rate of growth and therefore with dietary lysine content. These results suggest that HP is correlated with the first limiting amino acid rather than CP content. However, statistical significance disappeared or was less when these measurements were adjusted for body-weight. At higher ambient temperature, this interaction could be shown more clearly.

Chapter 5

EFFECT OF CONSTANT CRUDE PROTEIN AND VARYING LYSINE ON HEAT PRODUCTION IN HIGH AMBIENT TEMPERATURE

5.1. Introduction

Animals lose heat by different routes. The rate at which heat is lost depends on the difference in temperature between the body surface and its environment. A thermoneutral environment is one in which the animal does not need to increase energy expenditure to either warm or cool the body. The critical temperature is usually defined as the point at which an animal must increase its heat production to prevent body temperature from falling or increase the rate of heat dissipation to prevent body temperature from rising. Heat production is affected by factors which require energy, such as maintenance (the energy required for sustaining the basic processes in the body). Other factors affecting energy expenditure are thermoregulation (the energy required for keeping animal at normal body temperature), activity and food intake. Food intake is expected to decrease as ambient temperature increases since the bird's heat production is increased by activity of feeding and metabolism caused by digestion and assimilation of food. This increased heat production has been referred to as heat increment. Heat increment after food consumption and during digestion depends on the chemical composition of the diet. In the literature, there are several examples of less ideal compositions that increased heat increment and this may exacerbate a heat stress situation. Heat increment is much larger when protein is a source of energy than if

carbohydrates or fat are the source of energy. Heat increment for protein is much greater when the animal is at high ambient temperature than when it is at a low temperature (Musharaf and Latshaw, 1999). Heat increment from protein catabolism can be reduced by improving the amino acid balance (Austic, 1985).

In the previous experiment, heat production per bird increased significantly ($P<0.05$) with rate of growth and therefore with dietary lysine content (See Section 4.3.3.). However, the significance disappeared when the measurement was adjusted for body weight although a trend still remained. At temperatures below thermoneutral, any additional energy available may also be used for thermoregulation, although the experiment of MacLeod (1997) was also performed at 20 °C and demonstrated a wide range of heat production in relation to lysine intake and rate of protein accretion. To prevent the masking of dietary effects by thermoregulatory demands, this experiment was performed at a higher ambient temperature. At higher temperatures, it is possible that a further interaction may occur: any additional thermogenic effect of the diet may lead to reduced food intake if the bird is unable to dissipate additional heat.

The hypothesis being tested here was that there would be a clearer relationship between HP and the first limiting amino acid at higher ambient temperature without the effect of thermoregulation. This is because the heat produced by the metabolism of food can potentially replace thermoregulatory heat production.

5.2. Materials and Methods

Detailed procedures and measurements were the same as the previous experiment in Chapter 4 except for the environmental temperature (30 °C). At 21 d of age, chicks were randomly allocated to calorimeter chambers and kept for 7 d of acclimatisation before ambient temperature was increased to 30 °C. The experimental diets were formulated to the same specification used in the previous experiment (varying lysine and constant CP concentration; see Section 4.2).

5.3. Results

5.3.1. Food intake, water intake, excretion and growth

The results are summarized in Table 5.1. Food intake was affected by lysine concentration at high ambient temperature whether or not both values were adjusted for body weight. It increased significantly by 48% in Diet 14 compared with Diet 11 as lysine concentration increased ($P<0.001$).

Water intake was also significantly affected, along with food intake, by lysine concentration ($P<0.001$). It increased by up to 150% in the birds fed on Diet 14 compared with the birds fed on Diet 11.

Body-weight gain was also significantly affected by lysine concentration ($P<0.001$). The greatest body-weight gain occurred on high lysine diets (Diet 14 and 15) and there was a 3.2-fold range in weight gain with variation of dietary lysine

concentration (Table 5.1 and Figure 5.2). Body-weight gain was closely correlated with lysine intake (r=0.907, $P<0.001$; Figure 5.3).

Food intake, along with water intake, body-weight gain and dry matter excretion, was maximal on Diet 14 (12 g lysine/kg diet).

Table 5.1 *Effect of lysine concentration on intake, growth, FCE and excretion (dry matter; DM) at high ambient temperature*

Diet No.	11	12	13	14	15	SED	*P*
Lysine (g/kg)	6.0	8.0	10.0	12.0	14.0		
Food intake (g/bird.d)	63.4	96.1	92.9	114.2	105.8	5.92	<0.001
Food intake (g/kgW $^{0.75}$.d)	74.3	98.9	96.8	110.3	100.5	5.55	<0.001
Water intake (ml/bird.d)	93.0	246.5	193.5	278.0	264.0	22.15	<0.001
Water intake (ml/W$^{0.75}$.d)	108.9	254.6	201.1	270.3	249.8	26.57	<0.001
Water : Food ratio	1.48	2.57	2.08	2.52	2.51	0.35	0.022
Wt gain (g/bird.d)	21.5	55.8	51.1	72.7	71.2	4.99	<0.001
FCE	0.34	0.58	0.56	0.64	0.67	0.04	<0.001
Excretion, DM (g/bird. d)	20.1	26.5	28.5	33.3	30.7	2.80	0.006
Excretion, DM (g/kgW$^{0.75}$.d)	23.6	27.2	29.7	32.1	29.1	2.44	0.045

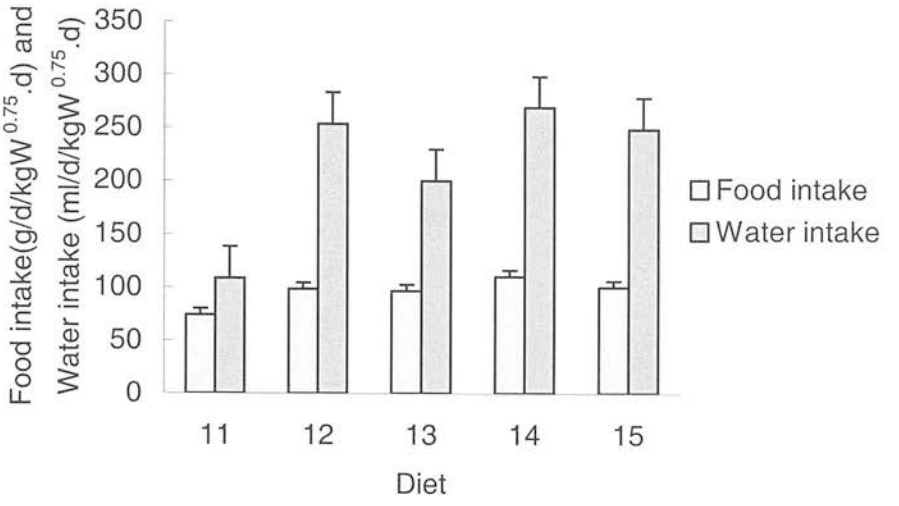


Figure 5.1 *Effect of lysine concentration on food intake and water intake at high ambient temperature. Lysine concentration of diets (g/kg) : 11; 6.0, 12; 8.0, 13; 10.0, 14; 12.0, 15; 14.0. Standard error bars were calculated from the pooled estimate of the results.*

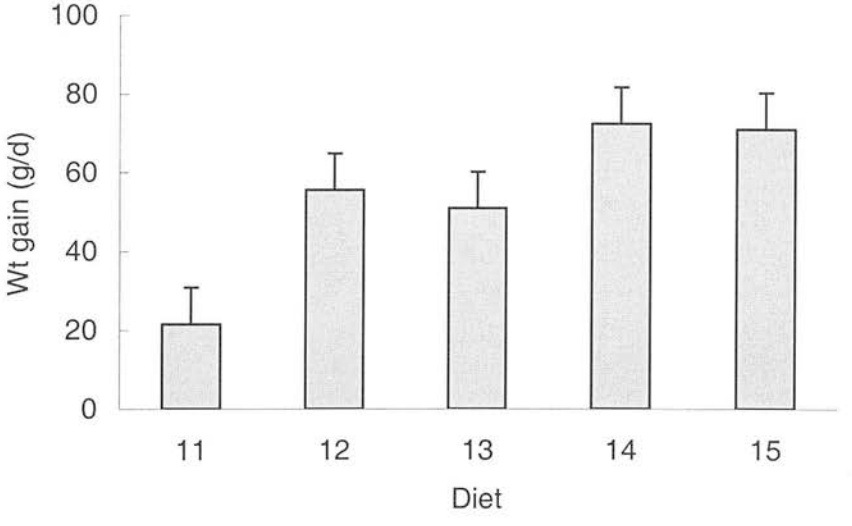


Figure 5.2 *Effect of lysine concentration on daily body-weight gain at high ambient temperature. Lysine concentration of diets (g/kg) : 11; 6.0, 12; 8.0, 13; 10.0, 14; 12.0, 15; 14.0. Standard error bars were calculated from the pooled estimate of the results.*

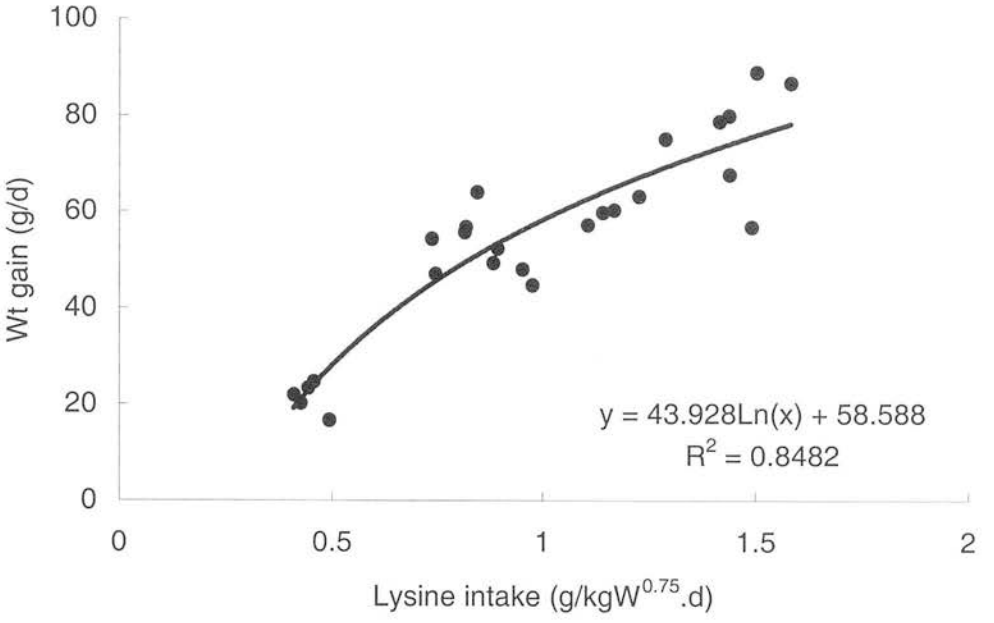


Figure 5.3 *The relationship between lysine intake and body-weight gain at high ambient temperature*

5.3.2. Nitrogen utilisation

Table 5.2 shows the effect of lysine concentration on N utilisation. Lysine concentration had a significant effect on N intake (P=0.005). N loss was not significantly different between diets but there was a tendency to increase as lysine concentration increased. N retention, therefore, was significantly different between varying lysine diets (P<0.001). N retention was highest in the birds fed on diet 14 and the increase was by 107% compared with diet 11, the lowest lysine diet. Efficiency of N retention increased significantly as lysine concentration increased (P<0.001, Figure 5.4).

Table 5.2 *Effect of lysine concentration on nitrogen (N) intake, loss, retention and efficiency of N retention at high ambient temperature*

Diet No.	11	12	13	14	15	SED	*P*
Lysine (g/kg)	6.0	8.0	10.0	12.0	14.0		
N intake (g/bird.d)	2.79	4.09	4.01	4.80	4.55	0.251	<0.001
N intake (g/kgW$^{0.75}$.d)	3.17	4.13	3.95	4.35	4.14	0.254	0.005
N retention (g/bird.d)	0.97	2.04	1.69	2.42	2.42	0.111	<0.001
N retention (g/kgW$^{0.75}$.d)	1.04	2.02	1.54	2.06	2.12	0.212	0.001
N loss (g/bird.d)	1.82	2.05	2.32	2.37	2.13	0.195	0.094
N loss (g/kgW$^{0.75}$.d)	2.13	2.11	2.42	2.29	2.02	0.170	0.205
Efficiency of N retention	0.35	0.50	0.42	0.51	0.53	0.023	<0.001

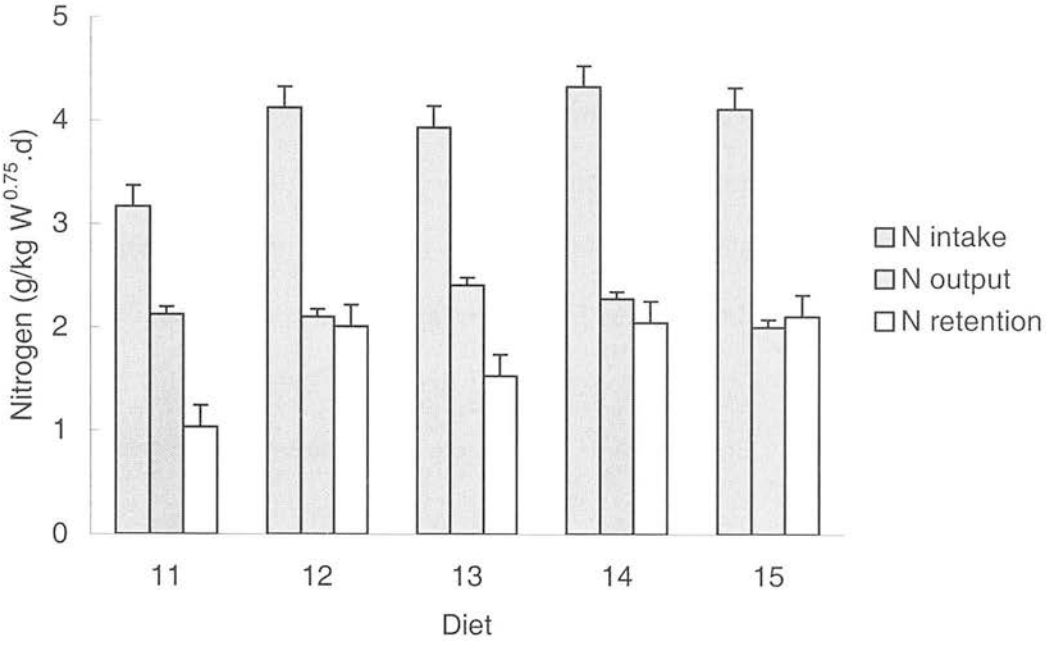


Figure 5.4 *Effect of lysine concentration on nitrogen intake, output and retention at high ambient temperature. Lysine concentration of diets (g/kg) : 11; 6.0, 12; 8.0, 13; 10.0, 14; 12.0, 15; 14.0. Standard error bars were calculated from the pooled estimate of the results.*

5.3.3. Heat production and energy utilisation

Table 5.3 shows the effect of lysine concentration on heat production and energy utilisation at high ambient temperature. Heat production (HP) per bird increased significantly with dietary lysine content, whether or not adjusted for body-weight ($P<0.001$; Table 5.3 and Figure 5.5). The trend was greater than in the previous experiment performed at 20 °C. It was also highly correlated with lysine intake ($r=0.686$, $P<0.001$; Figure 5.6). There was a 23% increase in HP in birds fed on Diet 14 compared with those fed on Diet 11. Birds fed on Diet 15 showed lower HP than the birds on lower lysine diets.

Table 5.3 *Effect of lysine concentration on heat production and energy utilisation at high ambient temperature*

Diet No.	11	12	13	14	15	SED	*P*
Lysine (g/kg)	6.0	8.0	10.0	12.0	14.0		
TME intake (kJ/bird.d)	850	1288	1245	1530	1417	79.3	<0.001
TME intake (kJ/kgW $^{0.75}$.d)	995	1326	1297	1478	1347	74.4	<0.001
E retention (kJ/bird.d)	287	621	505	741	664	61.4	<0.001
E retention (kJ/kgW $^{0.75}$.d)	337	640	526	715	634	61.0	<0.001
Heat production (kJ/bird.d)	514.6	659.3	670.8	764.8	722.2	24.2	<0.001
Heat production (kJ/kgW $^{0.75}$.d)	611.2	694.6	713.0	755.6	705.9	19.9	<0.001
CP retention (g / kgW$^{0.75}$.d)	6.0	12.7	10.6	15.2	15.1	0.7	<0.001
E retention as protein (kJ/bird.d)	143.2	302.7	250.7	359.1	358.8	16.5	<0.001
E ret as protein (kgW$^{0.75}$.d)	167.9	312.4	261.4	347.3	341.2	20.2	<0.001
E retention as fat (kJ/bird.d)	144	319	254	382	306	48.4	0.004
E retention as fat (kJ/kgW$^{0.75}$.d)	169	328	265	367	292	46.5	0.011

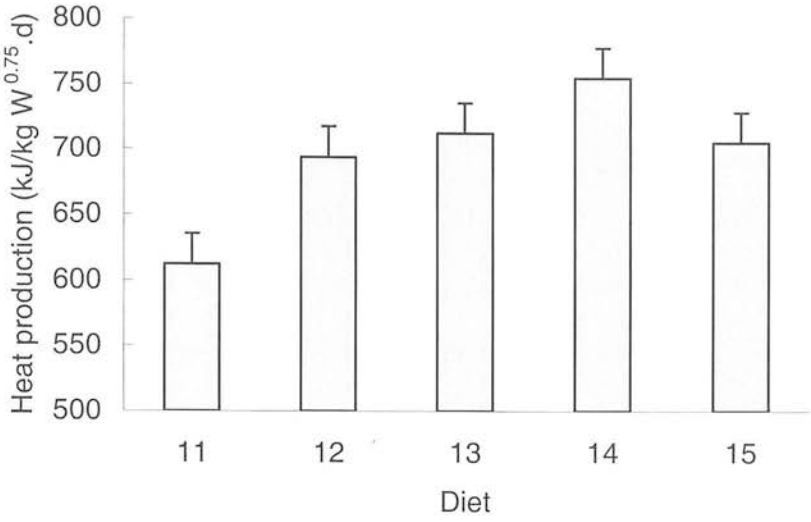


Figure 5.5 *Effect of lysine concentration on heat production at high ambient temperature. Lysine concentration of diets (g/kg) : 11; 6.0, 12; 8.0, 13; 10.0, 14; 12.0, 15; 14.0. Standard error bars were calculated from the pooled estimate of the results.*

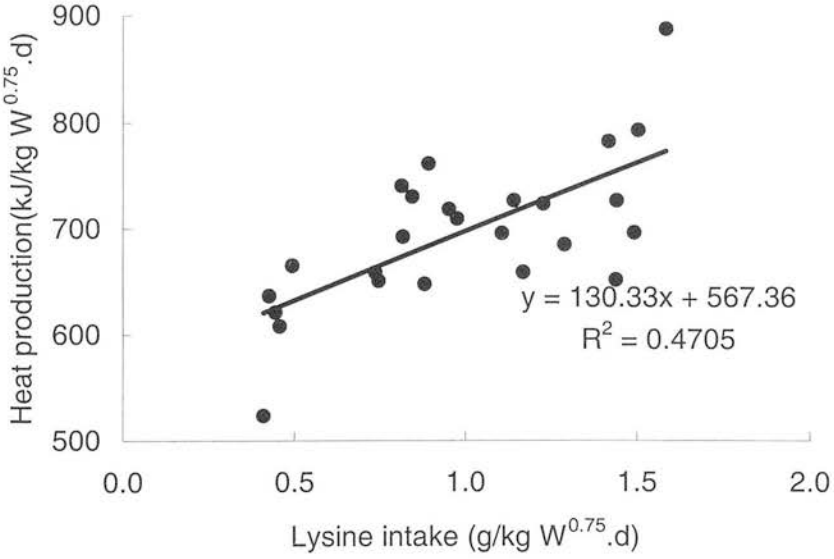


Figure 5.6 *The relationship between lysine intake and heat production at high ambient temperature. Standard errors are showd in square brackets. HP = 130.3[28.8]I_{Lys} + 567.4[30.4], (P<0.001)*

5.4. Discussion

5.4.1. Food intake, water intake and growth

Average food intake by birds at high ambient temperature was depressed by about 9% compared to that of birds under moderate temperature in the previous experiment. It is well known that higher environmental temperature decreases food intake in birds (*e.g.* Adams *et al.*, 1962a; Farrell and Swain, 1977; Geraert *et al.*, 1993). These authors also reported that growth rate is depressed as a result of the reduction in food intake. Above the thermoneutral zone, the body temperature rises and so food intake decreases in order to reduce the heat production associated with feeding, digestion, absorption and metabolism and to prevent an excessive increase in body temperature (Forbes, 1995). In this experiment, average daily body-weight gain was reduced by 20% compared to that of the previous experiment, performed at 20 °C (Figure 5.7). The reduction in growth rate as temperature increases above the optimum temperature has been reported by many researchers (*e.g.* Adams *et al.*, 1962b; Cowan and Michie, 1978; Cahaner and Leenstra, 1992; Alleman and Leclercq, 1997; Temim *et al.*, 1999). For broilers in the late period of growth, this optimum temperature for efficiency was found to be 21°C (Vo *et al.*, 1978). Adams *et al.* (1962b) examined diets containing increasing CP from 140 to 230 g/kg in broiler kept at 21 and 32 °C and suggested that even when the intake of protein was the same at both temperatures the birds at the higher temperature still grew more slowly than the birds at moderate temperature. McNaughton *et al.* (1978) examined the effects of environmental temperature on the growth responses of cockerels given different levels of lysine. Weights were maximal for 2-wk-old cockerels given 1.05% dietary lysine at both moderate (23.9 °C) and high temperature (29.4 °C) although there was no significant difference in mean body weight between the two

environmental temperatures. However, 4-wk-old cockerel weights were maximal when they were fed either 1.0% lysine diet in cool environment (15.6 °C) or 0.95% lysine diet in the warm environment (29.4 °C) and there was a significantly larger growth rate in birds kept at the lower temperature. However, the findings of March and Biely (1972) are different from the results with broilers. They reported that reduced growth at high temperature occurred when a lysine-deficient diet was fed but not when a lysine- adequate diet was fed to White Leghorn cockerels.

Diet 11 (0.6% lysine) gave low food intake and growth as shown in the previous experiment (Table 5.1). The significantly inferior growth performance of birds fed on Diet 11 clearly indicated that this diet was deficient in lysine. As shown in the previous experiment, there was no significant difference between the 1.2% and 1.4% lysine diet in food intake and the growth and it seems to be within a plateau range. Tesseraud *et al.* (1996) reported that, whatever the age, lysine deficiency resulted in significant decreases in body weight, tissue protein content and tissue protein deposition, apparently because of reduced amounts of protein synthesized. The trends for food intake and weight gain to increase as lysine increased were similar to those of the previous experiment although average intake and growth were reduced at higher temperature. Again, the 1.2% lysine diet gave maximal performance in this experiment. The effect of the concentration of first limiting amino acid on growth and food intake has been discussed in the previous chapter (see Section 4.4.1).

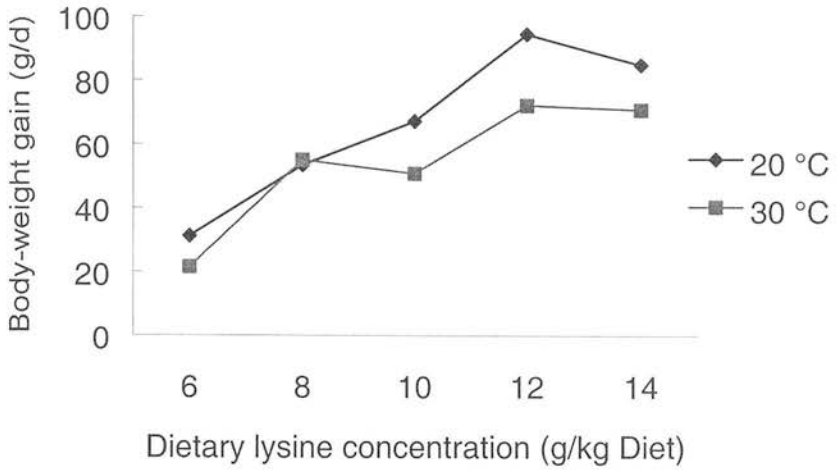


Figure 5.7 *Effect of two environmental temperatures (20 °C and 30 °C) on daily body-weight gain of birds given graded concentrations of lysine*

Average water intake was about 12% greater than in the previous experiment performed at 20 °C, although there was greater food intake with the birds kept at 20 °C. It seems that more water was required for evaporative cooling. Sykes (1977) reported that evaporation increased from about 50 to about 110 g/d when temperature increased from 20 to 30 °C. In general, water intake is correlated with food intake as shown in this experiment. However, over a certain range, although food intake decreased as ambient temperature increases, water consumption was still similar to that of birds which were kept at moderate temperature. This consumption can be markedly increased when the temperature rises above a critical level (Duke, 1986). Savory (1986) reported that water intake was nearly twice as much per day at 32 as at 8 or 20 °C and water consumption did not increase markedly until ambient temperature exceeded 30 °C. Waibel and MacLeod (1995) found that water intake

was greater when turkeys were kept at 29 than at 16 °C. They attributed this to the need for more water for respiratory evaporative cooling.

5.4.2. Nitrogen utilisation

N intake and N loss were lower than in the previous experiment at moderate temperature as a result of reduced food intake. N intake, N retention and efficiency of N retention were all significantly affected by dietary lysine concentration. Mean N retention decreased by about 25% compared with previous experiment. Farrell and Swain (1977) reported that N retention was maximal at the intermediate temperature (22 and 16 °C) and at the high temperatures the lower retention was associated with a reduced food intake. Temim *et al.* (1999) investigated the effects of high ambient temperature on performance and nitrogen retention. They reported that high-temperature-related growth reduction was associated with decreased nitrogen retention (-30 to -35%), which could not be explained by lower feed intake alone. At 32 °C, providing a high protein diet significantly increased weight gain and feed efficiency and slightly improved whole body protein deposition.

5.4.3. Heat production and energy utilisation

Mean heat production of all diet groups was about 20% lower at 30 °C in comparison with the experiment at 20 °C. Many reports show that decreasing temperature caused a linear increase in heat production. Greater heat production (kJ/d) with decreasing temperature from 35 to 2 °C was found in starving birds by Farrell and Swain (1977). Similar results were reported when ambient temperature ranged between 5 and 34 °C and heat production was corrected to the same body weight (Klandorf *et al.*, 1981).

This increase in heat production was as a result of the increase in energy demand and the rate of energy transformation. The effect of elevated temperature on heat production when animals are fed was also studied. A reduction in heat production (kJ/d) with increasing temperature was reported by Davis *et al.*, (1973) and Farrell and Swain (1977) and when corrected to the same body weight by Klandorf *et al.* (1981) and MacLeod (1990, 1992).

It is probable that a reduction in food intake by 9% affected the heat production. Food intake is known to change heat production. Heat production (kJ/kg $W^{0.75}$) is greater when birds are fed than when they are fasted. These results were confirmed by Klandorf *et al.* (1981) and by MacLeod (1990, 1992). The level of food intake also affects heat production. MacLeod *et al.* (1979) reported that hens and cockerels with food intake restricted to 80% of *ad libitum* had reductions in heat production of respectively 34% and 28% per bird per day when compared with the control groups. Wiernusz and Teeter (1993) reported that heat production and body temperature increased with food intake at 24 °C in broiler. The reduction in heat production as a result of the reduction in food intake was confirmed recently by Zhou and Yamamoto (1997).

There was a highly significant difference ($p<0.001$) between treatments as lysine concentration increased, reinforcing the statistically insignificant trend at 20 °C. The results agree with MacLeod's report (1997) which stated that heat production was strongly correlated with protein accretion, which in turn was strongly associated with lysine intake, not total protein intake.

Energy retention is mainly composed of protein and fat which are retained in the body. As a result of greater food intake in moderate temperature than in high temperature, more protein will be eaten. Also because elevated ambient temperature had no effect on protein retention efficiency (MacLeod, 1990, 1992) a greater amount of protein will be retained in the bodies of those birds which are kept at moderate temperature. Swain and Farrell (1975) reported an increase in protein retention efficiency with increasing temperature from 5 to 34 °C. They attributed this increase to the reduction in nitrogen excretion which was measured during starvation as temperature increases. However, the same authors (Farrell and Swain, 1977) reported a decrease in protein efficiency when temperature increased from 16 and 22 to 30 and 35°C. They related this decrease in efficiency at temperature to the reduction in food intake.

The influence of high temperature on energy retention as fat has also been investigated by many scientists. Elevating temperature from 18 to 33 °C increased the proportion of fat (Olson *et al.*, 1972; Kubena *et al.*, 1972; Howlider and Rose, 1987) while Mickelberry *et al.* (1966) noted no effect of increasing temperature from 21 to 29 °C on the ratio of fat in broilers at 5 weeks old. Sinurat and Balnave (1985) also found that high temperature (25 to 25 °C) had no significant effect on abdominal fat content when birds were compared with those reared at lower temperature (18 to 26 °C), although slightly more was noted in the birds which were kept at high temperature. Energy retention as fat was increased 40 % when compared to that of the previous experiment at lower ambient temperature. At high temperature, less energy is required for thermoregulation and this leads to increased storage of energy as body fat.

Total energy retention was found to be less at high temperature than at moderate temperature in report by Farrell and Swain (1977) and MacLeod (1990, 1992). This was attributed to the reduced food intake. However, energy retention at high temperature was greater than at moderate temperature in this experiment. Similar results were reported by Kleiber and Dougherty (1934). They noted energy retention (kJ/d) at 32 °C was greater than at 21 °C. They attributed this to the additional energy required to maintain body temperature at lower environmental temperature. Mean energy retention of birds at high temperature was slightly higher than those at moderate temperature in present experiment.

5.5. Conclusion

Similar to the results of the previous experiment, food intake, growth rate and N utilisation efficiency were significantly increased as lysine concentration increased. HP was also affected significantly by dietary lysine content whether or not adjusted for body-weight. The trend was greater than in the previous experiment performed at 20 °C.

Three calorimetry experiments including one in this chapter confirmed that HP is strongly correlated with the first limiting amino acid rather than total CP content. It can be concluded that there was little indication of regulatory diet-induced thermogenesis when an imbalanced amino acid mixture or excess amino acids were fed.

Chapter 6

EFFECT OF PROTEIN QUALITY ON GROWTH RATE AND CARCASE CHARACTERISTICS

6.1. Introduction

Protein quality is defined as the extent to which the diet can meet the essential amino acid needs of the animal (see Section 1.2.2.1). Amino acid requirements of animals depend on various factors such as sex, strain, diet, age, body composition and environment. Determining amino acid requirements at each stage of growth is not easy and has to be modified each time when growth characteristics change substantially. An alternative approach is to first establish the ideal protein requirement for the bird relative to body weight and then periodically determine the requirement of one of the amino acids. Ideal protein can be defined as the perfect profile or balance in terms of dietary concentrations among the amino acids. A concept of ideal protein was first published in the late fifties and early sixties (Dean and Scott, 1965). This concept basically states that the amino acid requirements are relative to each other because they depend on the amino acid composition of total body protein. Baker and Han (1994a) reported ideal protein estimates for the chicken relative to lysine requirements because lysine most influences growth and it is relatively simple to determine in growth trials. Ideal protein estimates for broiler, turkey and pig were also reported by Firman and Boling (1998).

Both quantity (CP concentration) and quality (amino acid balance) of dietary protein affect the body composition of chickens (Khalil *et al.*, 1968). For example, diets

with low energy:protein ratio promote lean broiler carcases (Donaldson *et al.*, 1956; Thomas and Combs, 1967). It is possible that diets with a very low energy:protein ratio promote lean broilers by restricting energy consumption (Bartov, 1979). Excretion of surplus amino acids may also require metabolic energy that is, therefore, not available for fat synthesis (Buttery and Boorman, 1976). Dietary protein quality reflects a balance of amino acids required for maximum growth and lean tissue synthesis and the limiting amino acid levels in a protein source (Fisher *et al.*, 1959).

Most studies investigating the relationship between protein and body composition have been focused on reducing dietary protein level in broiler diets below the recommendations and supplementing these diets with essential amino acids, mainly lysine and methionine. Such diets usually support adequate growth but increase fattening (Lipstein *et al.*, 1975; Moran *et al.*, 1992; Deschepper and De Groote, 1995), and reduce, or do not affect, the yield of meat (Moran *et al.*, 1992 and Deschepper and De Groote, 1995, respectively). The effect of diets containing higher protein levels than recommended, on the yield of carcase and breast meat, in contrast to their effect in decreasing fattening (Bartov, 1996) has rarely been investigated. Negative effects on performance were observed when diets containing extremely high protein levels were used (Holsheimer and Veerkamp, 1992).

In the previous experiments, various diets differing in amino acid balance were tested in relation to energy and nitrogen metabolism. This experiment was performed to investigate, on a larger scale, the effect of amino acid balance and protein quality on growth rate, breast meat yield and fat deposition. The hypothesis tested in this experiment was whether imbalanced high-protein diets could accomplish similar

performance as well-balanced protein diet. It was also important to test the results of the calorimetry experiments under commercial-type conditions.

6.2. Materials and Methods

Four hundred male broilers (Ross 308) were used. Five replicate pens of 20 birds were grown to 42 d on each diet. Four diets containing 13.4 MJ/kg of TME were formulated (Table 6.1). An "ideal protein" diet was formulated to provide NRC requirements of amino acids at the minimum protein content, allowing supplementation with lysine, methionine, threonine, tryptophan and arginine. The extreme high protein diet was formulated to provide the same amino acid intakes without supplementation. However, total lysine content in the highest protein diet was increased by 5% to allow for the greater digestibility of the supplement in the "ideal" and intermediate diets. This correction was calculated as follows. In the "ideal protein" diet, 3.5/11 of total lysine is crystalline (= 0.32). Adjusted by 9.5/12 for hydrochloride content, this becomes 0.25. On average, crystalline lysine is 20% more digestible than lysine from natural raw materials. This means that total lysine in the "ideal protein" diet is 0.20 x 0.25 more digestible (5%). A similar calculation for methionine leads to increasing total methionine by 9% in the "high protein" diet. The lowest diet and the highest diet were formulated using FORMAT NC software and two intermediate diets were calculated by linear interpolation.

Body weights were recorded at 1, 21 and 42 d. Food intake was measured between d 1 and 21 and between d 21 and 42. At d 42, two median birds from each pen were killed and dissected for breast meat yield and fat pad weight. Food and water were

available *ad libitum* during the experimental period. Temperature was reduced from 30 ° C to 20 ° C over the first 3 weeks and lighting was on a 23h light : 1h dark pattern.

The diets were allocated to pens in a randomised block design. In all cases, pen means werw used as the experimental units. One-way analyses of variance fitting the effect of diet were carried out using GenStat according to following model.

$$X = \text{Mean} + \text{Deit} + \text{Block} + \text{Residual}$$

Table 6.1 *Specifications and ingredients of experimental diets varying in CP content used in growth trial on a larger scale*

Diet	1	2	3	4
	Ideal protein			High protein
TME [1] MJ/kg	13.4	13.4	13.4	13.4
CP [2] g/kg	205	235	265	294
E : P ratio	0.065	0.057	0.051	0.046
Lysine concentration g/kg	11.0	11.2	11.4	11.6
Lysine : CP ratio	0.054	0.048	0.043	0.039
Ingredients (g/kg)				
Choline chloride	0.30	0.30	0.30	0.30
Dicalcium phosphate	14.00	12.25	10.49	8.74
Fish meal	29.00	38.54	48.08	57.62
Limestone flour	8.57	8.16	7.75	7.34
Maize gluten meal (60% CP)	40.3	76.9	113.4	150.0
Sodium chloride	3.42	3.17	3.04	2.80
Soya bean meal (48% CP)	160.5	177.7	194.9	212.0
Soya oil	50.8	47.0	43.2	39.4
Vitamin / mineral supplement	5.0	5.0	5.0	5.0
Wheat meal	665.0	610.9	556.8	502.8
Pellet binder	10.0	10.0	10.0	10.0
Titanium dioxide	4.0	4.0	4.0	4.0
Arginine	1.85	1.24	0.62	0.00
Lysine	3.46	2.30	1.15	0.00
Methionine	2.52	1.68	0.84	0.00
Threonine	1.28	0.86	0.43	0.00

[1] TME : true metabolisable energy, [2] CP : crude protein

6.3. Results

Table 6.2 shows the results. During the first 3-week period, food intake decreased as CP content increased up to 26%. However, the highest protein diet showed higher food intake than others. There was no significant difference in food intake between diets. In general, there was a tendency for food intake on the intermediate diets to be slightly lower but this produced statistical significance only up to 3 weeks.

Weight gain did not show significant differences between diets but there was a slightly lower weight gain on intermediate diets. This effect did not reach significance even in the first 3 weeks of the experimental period. Weight gain was near commercial target. Food conversion efficiency (FCE) was also similar in all treatments.

Breast muscle yield, in absolute and relative terms, was highest in diet 1 (ideal protein) but this effect did not attain statistical significance. Abdominal fat pad weight did not differ significantly between diets but tended to be lowest in diet 4 (high protein).

Table 6.2 *Effect of protein quality on growth and carcase characteristics of growing broilers*

Diet		1	2	3	4	SED	*P*
CP (g/kg)		205	235	265	294		
FI (g/b)	0-3wk	1077	996	983	1097	43.2	0.05
	3-6wk	3225	3125	3130	3157	92.4	0.692
	0-6wk	4302	4121	4113	4254	104.8	0.232
FCE	0-3wk	0.70	0.70	0.71	0.71	0.016	0.840
	3-6wk	0.56	0.58	0.57	0.57	0.011	0.444
	0-6wk	0.60	0.61	0.60	0.61	0.010	0.517
Wt (g)	0wk	36.9	37.4	37.1	36.6	0.381	0.291
	3wk	788	739	735	816	30.1	0.055
	6wk	2597	2550	2511	2610	48	0.206
Wt gain (g)	0-3wk	751	701	698	779	30.2	0.054
	3-6wk	1809	1811	1776	1794	38.2	0.781
	0-6wk	2560	2512	2474	2573	48	0.203
Breast muscle wt (g)		455.4	406.2	439.0	403.7	26.8	0.17
Breast muscle proportion		0.169	0.156	0.163	0.152	0.009	0.279
Abdominal fat wt (g)		35.5	35.1	39.9	28.5	4.72	0.137
Abd. fat proportion		0.0133	0.0135	0.0148	0.0106	0.0018	0.146

6.4. Discussion

The results show that there were no significant effects of protein content on food intake and growth rate when lysine content was kept constant. The effects of amino acid imbalance are acknowledged to be more severe at deficient protein intakes (Harper *et al.*, 1970). Sklan and Plavnik (2002) reported that increasing CP from 182 to 242 g/kg resulted in a linear decrease in food intake while weight gain and feed efficiency changed quadratically with a smaller positive effect at the highest protein intakes. Decreases in food intake with increasing CP level have also been reported previously (Parsons and Baker, 1982; Fancher and Jensen, 1989; Smith and Pesti, 1998). In the present experiment, it seems that the first limiting amino acid had the most important effect on food intake and growth. This result agrees with what has been shown in the previous calorimeter experiment in Chapter 3, which showed no significant difference in food intake although growth rate tended to be lower as CP increased. MacLeod (1990) found that there was no effect of CP concentration on food intake when he fed diets ranging from 130 to 260 g/kg of CP. Shariatmadari and Forbes (1993) also reported that there was no significant difference in food intake and growth rate between diets ranging from 172 to 280 g/kg CP.

Dietary protein concentration or E:P ratio also affect carcase characteristics of broiler chicks. Diets lower in protein than recommended (NRC, 1994) reduced the yield of meat (Moran *et al.*, 1992) and increased fattening (Moran *et al.*, 1992; Bartov, 1996). Some previous studies have shown that excess CP or relatively low E:P ratio reduced abdominal fat pad weight (*e.g.* Bartov, 1989, 1996; Bartov and Plavnik, 1998). The very recent report of Sklan and Plavnik (2002) also observed the negative correlation between CP level and fat abdominal fat deposition, whether the essential amino

acids:CP ratio was fixed or varied. It is not clear whether this was due to the decreased food intake or to metabolic changes. They suggested that when CP or amino acids are given in excess, energy is not deposited as protein but can be used for fat synthesis after deamination. In the present experiment, there was no significant difference in abdominal fat pad deposition and breast meat yield although it tended to be the lowest at the highest protein diet. The results from this experiment are in agreement with those from Chapter 3, in that reducing total crude protein content while maintaining the intake of essential amino acids reduced nitrogen losses without showing any significant detrimental effects on growth rate, body composition or breast meat yield.

6.5. Conclusion

This experiment was performed to test the effect of amino acid balance and protein quality on the performance of birds on a larger scale. The result indicated that there was no significant effect of a high protein diet on growth rate or carcase composition. Overall, it seems that control of energy intake or the first limiting amino acid took priority over control of food intake.

Chapter 7

DIET SELECTION FOR PROTEIN QUALITY BY GROWING BROILER CHICKEN

7.1. Introduction

Animals have a tendency to avoid limiting their diet composition to only one food when given a free access to two or more foods of varying nutrient composition (Forbes, 1995). Thompson (1980) suggested that this tendency may coincidentally result in animals meeting their various dietary requirements. This proposition is rather simplistic because it implies that animals select foods to meet their requirement by chance. From the findings of Rodgers and Rozin (1966), it is obvious that diet selection is partly a function of diet composition and nutritional status of an animal.

Birds select an optimum diet between foods of different protein content to provide themselves with a diet which is close to the optimal for growth. Factors that influence dietary choices by chickens include genotype, age, physiological state, and prior experience (*e.g.* Kaufmann *et al.*, 1978; Rose and Kyriazakis, 1991; Shariatmadari and Forbes, 1993; Forbes, 1995). When choice diets provided more than or less than the optimum concentration of protein, the preferred diet of growing pig was that which allowed growth at a rate similar to those fed a single diet that was assumed to be optimal (Kyriazakis *et al.*, 1990). When chickens were offered diets either above or below optimal protein content, they consumed predominantly the diet closest to optimum (Shariatmadari and Forbes, 1993), a result that agrees with the

statement by Emmans (1977) that when formulations of two diets were such that no mixture of them was of adequate composition then the animal would select in a way to minimize the inadequacy or excess. Holcombe *et al.* (1976) showed that layers chose a higher proportion of the low protein diet, whereas when the choice was between diets adequate or higher in protein they chose more of the adequate diet. This observation was confirmed in broilers by Shariatmadari and Forbes (1993) who reported reduced protein intake in choice situations involving diets with very low and high protein contents.

Decreasing food intake has been related to abnormal proportions of amino acids. Imbalances caused by manipulating individual dietary amino acid levels have been defined as a form of indispensable amino acid deficiency (Harper *et al.*, 1970). The absence of threonine or isoleucine reduced food intake of rats by more than 50 % which resulted in continuous weight loss. Dietary preference for amino acid balanced diets over imbalanced diets has been reported with rats. There is not much information on diet selection of poultry with few investigations involving specific amino acids (Newman and Sands, 1983; Edmonds and Baker, 1987; Steinruck *et al.*, 1990b; Roth *et al.*, 1990) and essentially none on genetic variation for diet choice. Picard *et al.* (1993) demonstrated that, when effects of raw materials were removed, broilers were able to distinguish between a balanced diet and one deficient in lysine, methionine, and tryptophan. Noble *et al.* (1993) confirmed this by measuring in two genetic lines, discriminatory food intake, and growth responses to diets varying in some amino acids. Newman and Sands (1983) investigated the choice by young layer chicks between a low-lysine diet and one with excess of lysine. Although the birds consumed some of the supplemented food it was not enough to maintain a growth rate as high as those in a control group which were given a single adequate

diet. However, these chicks showed definite preference for the adequate lysine diet, which implies sensory recognition of lysine by birds.

In the previous experiments in this thesis, the effect of amino acid balance was tested in terms of energy and nitrogen metabolism. In the first experiment in this chapter, free-choice feeding was performed with similar diets to investigate the sensitivity of the broiler chicken to its diet on the basis of amino acid balance, especially related to lysine concentration. The effects of lysine concentration on food intake and growth rate were also compared to the results of previous experiment. In the second experiment, an ideal protein diet and a high protein diet similar to those used in previous growth trial (Chapter 6) were used to investigate the sensitivity of the chicks to diets different in protein quality.

The hypothesis tested in this choice-feeding experiment was that birds could select between an adequate or balanced diet and a diet with an amino acid deficiency or excess.

7.2. Materials and Method

Experiment 1

Forty-eight male broiler chicks were used. The birds were reared in brooders for 20 days after hatching on a common starter diet. At 21 day of age, they were randomly allocated (in pairs) into cages fitted with two feeding troughs and a water container (Figure 7.1). Three diets with different lysine concentrations were formulated (Table 7.1 and 7.2). The lysine concentrations were 6 g/kg (low), 10 g/kg (moderate; NRC (1994) recommendation for 3-6 wk old broiler) and 14 g/kg (high) respectively. Other amino acids were maintained at concentrations 1.5 times the published recommendations, to ensure that no other amino acid became limiting. The diets were made by adding the supplements on the basal diet to minimise the palatability for certain feed ingredient. As supplemental lysine is removed, stepwise, it was replaced with 2X its weight of glutamic acid to keep diets isonitrogenous (Table 7.2). Birds were divided into 4 treatment groups. Group A was offered the choice between the low lysine diet and the moderate lysine diet. Group B was offered the low and high lysine diets. Group C was given the moderate and high lysine diets. Group D had only the moderate lysine diet, to act as the control group but the food was given in two separate troughs. All diets contained 300 g/kg of crude protein and 13.4 MJ/kg of true metabolisable energy. Foods and water were available *ad libitum*. Diets and fresh water were replenished daily. Two days were allowed for the birds to become accustomed to the experimental set-up. Food and water intake and body weight were measured daily thereafter, for 10 days. The environmental temperature was 22 °C and the birds were kept on a 23L:1D lighting cycle. Statistical significance of the choice was tested by one-sample t-test under the null hypothesis that the birds ate equal amounts of the two diets.

Table 7.1 *Formulation of basal diet in experiment 1*

Ingredient	g/kg
Wheat meal	468.8
Barley meal	86.8
Maize gluten meal (60% CP)	346.5
Maize oil	10
Choline chloride	0.3
Fish meal	9.6
Dicalcium phosphate	29.1
Sodium chloride	2.9
Limestone flour	15
Arginine	8
Methionine	1.4
Threonine	1.83
Tryptophan	0.8
Vitamin/mineral supplement	5
Pellet binder	10

Table 7.2 *Composition of supplements in experiment 1*

Diet	Supplements (g/kg of diet)		
	Lysine	Glutamic acid	Maize starch
Low	0	15.83	0
Moderate	5.0	7.92	2.91
High	10.0	0	5.83

Experiment 2

Twenty male boiler chicks were reared to 14 d in brooders on a standard starter diet. At 14 d they were randomly allocated to individual cages fitted with two troughs, one for each of the two diets. Formulations of the two diets are in Table 7.3. Diets and water were available *ad libitum*. Food and fresh water were replenished daily. Food intake and body weight were measured daily thereafter, for 14 days. The environmental temperature was 22 °C and the birds were kept on a 23L:1D lighting cycle. The data were analysed by one sample t-test and analysis of variance.

Table 7.3 *Formulation of experimental diets in experiment 2*

	Ideal Protein	High Protein
TME MJ/kg	13.4	13.4
CP g/kg	205	294
Lysine concentration g/kg	11.0	11.6
Choline chloride	0.30	0.30
Dicalcium phosphate	14.00	8.74
Fish meal	29.00	57.62
Limestone flour	8.57	7.34
Maize gluten meal (60% CP)	40.3	150.0
Sodium chloride	3.42	2.80
Soya bean meal (48% CP)	160.5	212.0
Soya oil	50.8	39.4
Vitamin / mineral supplement	5.0	5.0
Wheat meal	665.0	502.8
Pellet binder	10.0	10.0
Titanium dioxide	4.0	4.0
Arginine	1.85	0.00
Lysine	3.46	0.00
Methionine	2.52	0.00
Threonine	1.28	0.00

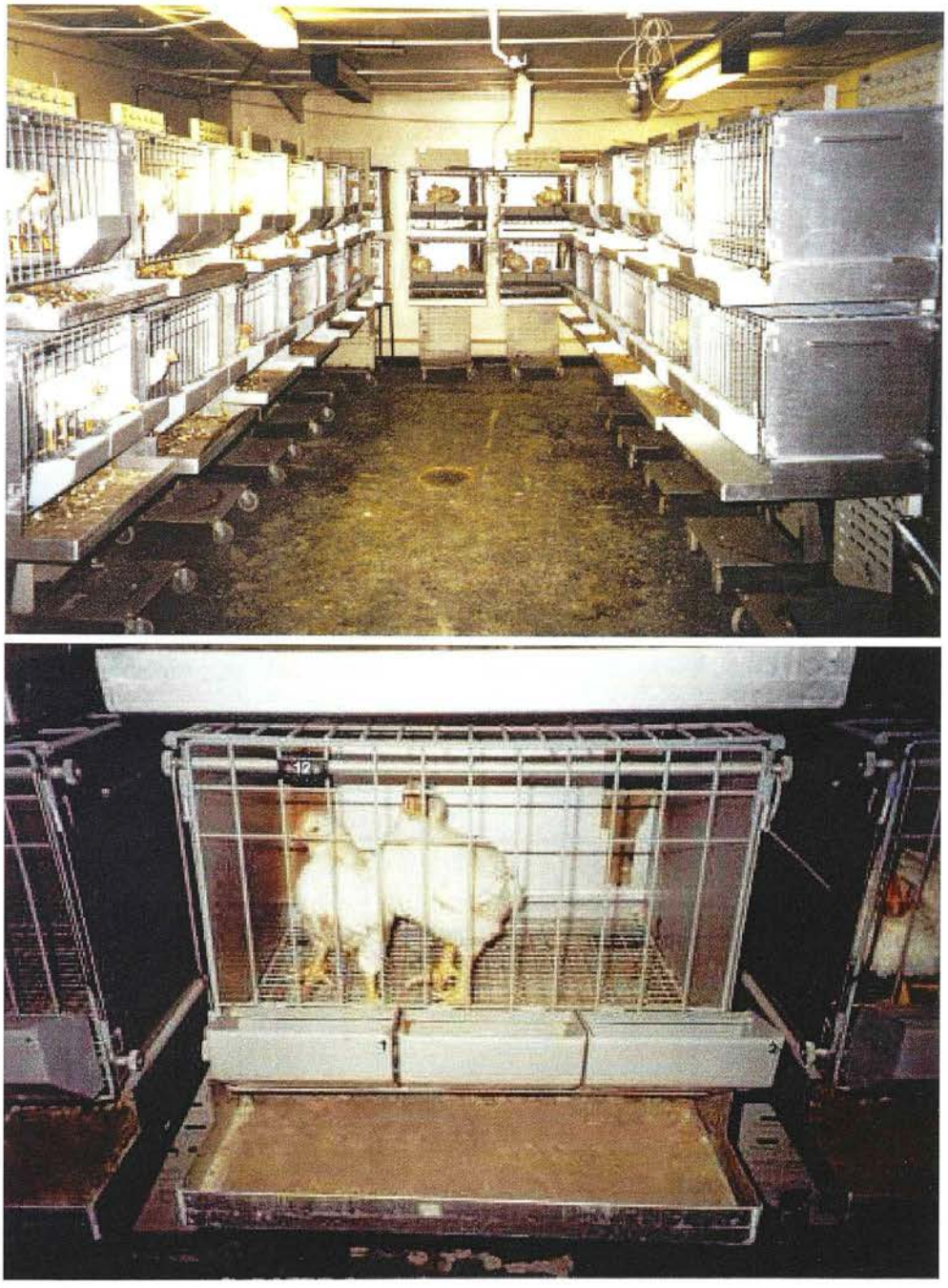


Figure 7.1 *Chickens allocated in pairs into cages fitted with two feeding troughs and a water container (centre)*

7.3. Results

Experiment 1

Over the whole period of the experiment, birds consumed some of each diet offered, but preference was shown for the moderate or high lysine diet over the low lysine diet. During the first 5-day period, group A showed a preference for the moderate lysine diet (Table 7.4). Group B also showed that the bird consumed more of the high lysine diet than the low lysine one but the tendency was not large enough to reach significance. In the second half of the period, the difference in selection reached highly significant levels in both group A and B ($P<0.001$). Both groups consumed about 2.5 times as much of the moderate and high lysine diets as of low lysine diet. In groups C and D, there was no significant difference in selection over the whole period of measurement.

Group A and B, which had a low lysine diet included in their choice showed lower total food and water intake and growth rate than the groups including high lysine diet (Table 7.5). Growth rate reflected total lysine intake (Table 7.6, Figure 7.2). Although the birds consumed some of moderate lysine diet in group A, it was not enough to produce a growth rate as high as those in control group D.

Table 7.4 *Selection between low, moderate and high lysine diets*

Group	A		B		C		D	
Lysine content	Low	Mod [1]	Low [2]	High [3]	Mod	High	Mod	Mod
Proportions of intake d1-d5	0.436	0.564	0.465	0.535	0.485	0.515	0.495	0.505
Proportions of intake d6-d10	0.300	0.700	0.281	0.719	0.521	0.479	0.493	0.507
Proportions of intake d1-d10	0.357	0.643	0.362	0.638	0.506	0.494	0.494	0.506
Differences from equality in proportions of intake d1-d5	0.064 (P=0.058)		0.036 (P=0.015)		0.015 (P=0.245)		0.006 (P=0.807)	
Differences from equality in proportions of intake d6-d10	0.201 (P=0.002)		0.219 (P<0.001)		-0.021 (P=0.238)		0.007 (P=0.785)	
Differences from equality in proportions of intake d1-d10	0.143 (P=0.002)		0.138 (P=0.001)		-0.006 (P=0.064)		0.006 (P=0.767)	

[1] Moderate ; 10 g/kg lysine (NRC recommendation), [2] Low ; 6 g/kg lysine,
[3] High ; 14 g/kg lysine
* Statistical significance of the choice was tested by one-sample t-test under the null hypothesis that the birds ate equal amounts of the two diets.

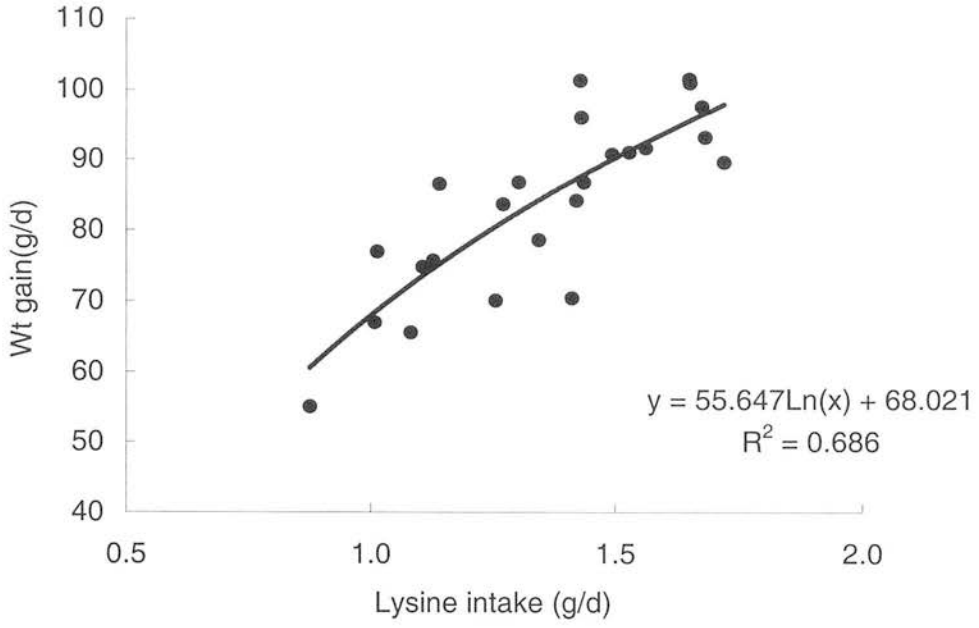


Figure 7.2 *The relationship between body-weight gain and lysine intake, including all diet choices in experiment 1*

Table 7.5 *Total food intake and water intake of birds (d1-d10)*

Group	A	B	C	D	SED	*P*
Total food intake(g/b/d)	143.0	139.8	159.9	157.5	6.45	0.003
Water intake(ml/b/d)	246.3	259.0	294.0	252.2	13.49	0.003

Table 7.6 *Daily lysine intake of birds (d1-d10)*

Group	A		B		C		D	
	L	M[1]	L[2]	H[3]	M	H	M	M
Lysine intake(g/b/d)	0.261	0.802	0.273	1.114	0.688	0.945	0.663	0.689
Total lysine intake	1.063		1.387		1.633		1.352	
SED	0.079							
P	<0.001							

[1] Moderate ; 10 g/kg lysine (NRC recommendation), [2] Low ; 6 g/kg lysine,
[3] High ; 14 g/kg lysine

Experiment 2

Table 7.7 shows the results of the experiment. During the first 7-day period, birds consumed more of the Ideal Protein diet than the High Protein diet (P=0.16). During the second half period, birds showed greater preference for the Ideal Protein diet (P<0.001). Birds consumed about 2.5 times as much of the Ideal Protein diet as of the imbalanced diet over the whole experimental period of 14 days.

Table 7.7 *Selection between ideal protein diet and high protein diet of birds*

	Protein quality choice	
	Ideal Protein	High Protein
Proportions of intake d0-d7	0.655	0.345
Proportions of intake d7-d14	0.718	0.282
Proportions of intake d0-d14	0.694	0.306
Differences from equality in proportions of intake d0-d7	0.155 (P=0.16)	
Differences from equality in proportions of intake d7-d14	0.218 (P<0.001)	
Differences from equality in proportions of intake d0-d14	0.194 (P<0.001)	

* Statistical significance of the choice was tested by one-sample t-test under the null hypothesis that the birds ate equal amounts of the two diets.

7.4. Discussion

In the first experiment, groups A and B showed a very similar degree of preference for the higher of the two lysine concentrations which they were offered. The selection became more significant with time. Both groups consumed about 2.5 times as much of the moderate and high lysine diets as of the low lysine diet. In group B, this led to a lysine intake equal to that of the control group (D). These results agree with Newman and Sands (1983) who reported significant preference for an adequate lysine diet over a low lysine diet. According to Murphy and King (1989), sparrows showed a preference even for a protein-free diet over a high-protein diet with a severe imbalance of amino acids. It is assumed that the birds learn that one food contains too little of an amino acid for its requirements and another doesn't. As mentioned in the literature review, Forbes (1995) stated that it is necessary to give a colour cue if the nutrient in question is only required in trace amounts and especially if it is colourless. Lysine is colourless and has little taste. In this experiment, however, the birds showed clear preference although colour cue was not used. It appears that birds learned the difference between the two foods primarily by their positions in the cage since the position of two troughs was not changed as the case of Newman and Sands (1983). Steinruck *et al.* (1990b) examined broilers given a choice between a complete diet and methionine-deficient diet (half the recommendation) chose predominantly the former, especially after they had been made methionine-deficient by prior feeding on the low-methionine diet. The same authors (1990a) investigated the effect on diet selection and growth of position of complete and methionine-deficient foods in the cage. Deficient birds were either given the two foods in the same position everyday for 36 days or the positions of the foods were reversed every 3, 6 or 9 days. Where the positions were unchanged there

was a 0.68 choice of the complete diet, but as the changes became more rapid so selection was less appropriate, being 0.65, 0.62 and 0.58 for the 9, 6 and 3-day change over birds, respectively. Uzu and Picard (1993) also examined the effect of location of feeders by choice feeding between a balanced diet and a methionine-deficient diet in laying hens and reported that when location was reversed, approximately 1 week was required for hens to again exhibit preference for the balanced diet. Forbes (1994) pointed out that some authors have misguidedly reversed the positions of two diets at frequent intervals in the hopes of avoiding positional bias and have seen poor diet selection as a result.

In groups C and D, there was no significant difference between selections over the whole period of measurement. In the case of group C, it appeared that the birds' sensitivity was not high enough to distinguish between moderate lysine and high lysine. This may have been caused by the high protein content of the diets, since the dietary protein concentration can affect the requirements for individual essential amino acids (Boomgaardt and Baker, 1971; Morris *et al.*, 1987; Mendonca and Jensen, 1989). Even among the published recommendations of lysine for broiler, there are some differences between reports (*e.g.* Holsheimer, 1981; Boomgaardt and Baker, 1973; Twining *et al.*, 1974). Therefore, the high lysine diet (14 g/kg) in this experiment might not be excessive for birds in this circumstance.

Groups A and B, which had a low lysine diet included in their choice, showed lower total food and water intake and growth rate than the groups including high lysine diet. The same tendency has been shown in the earlier calorimetry experiments reported in this thesis (Section 4.3.1). Growth rate reflected total lysine intake (Table 7.6, Figure 7.2). Although the birds consumed some of high lysine diet in group A, growth rate

was not as high as those in other groups (Figure 7.3). Newman and Sands (1983) showed similar results. They offered newly hatched layer chicks a choice between a low-lysine diet and excess-lysine diet. Although the birds consumed some of the supplemented diet it was not enough to maintain a growth rate as high as those in a control group which were given a single adequate food.

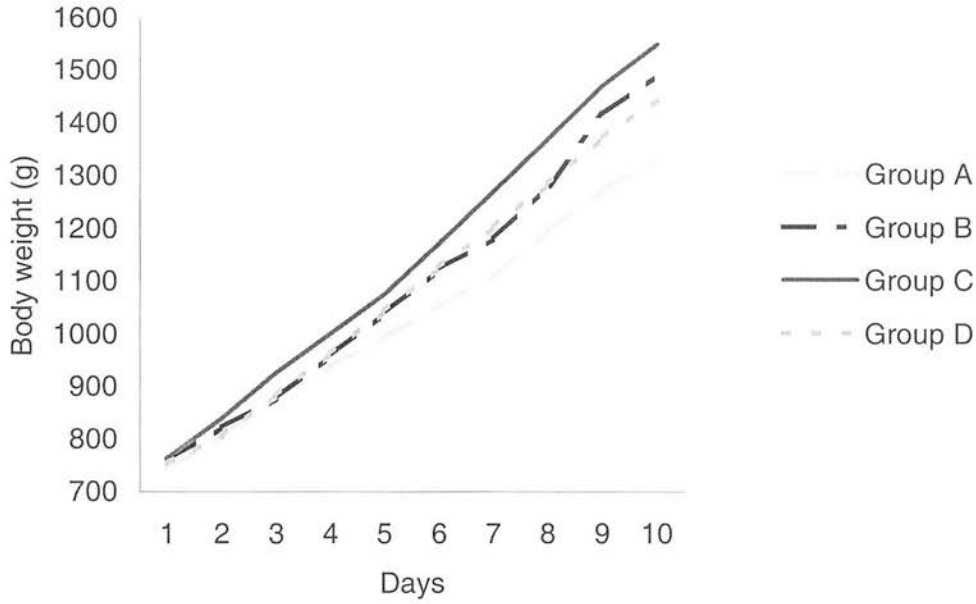


Figure 7.3 *Daily mean body weight of each group during experimental period in experiment 1*

The second experiment also confirmed the bird's ability to select an ideal protein diet to high-protein imbalanced diet. When given simultaneous choice of two diets, birds consumed 70% of the ideal-protein diet and 30% of the high-protein diet. Holcombe *et al.* (1976) have shown that layer chickens were able to select diets varying in their protein contents and to reject those diets in which the protein contents were far from

those required to match their requirements. Emmans (1991) stated that where the formulation of two diets is such that no mixture of them is of adequate composition then the animal will choose from them in such a way that the inadequacy or excess is minimised. Thus, birds offered two diets avoid the higher protein diet and thus avoid the excess protein intake. In the experiment of Shariatmadari and Forbes (1993), birds offered choices between adequate-protein diet and high-protein diet and low-protein diet and adequate –protein diet chose their intake almost entirely from the one closer to adequacy and rejected the extreme one.

Diets which are imbalanced in the amino acids absorbed from the digestive tract lead to metabolic disturbances and reduced food intake (Boorman, 1979). The reduction in food intake is directly proportional to the degree of amino acid deficiency or imbalance. This is thought to be due to the metabolic cost of deaminating the excess of those amino acids which cannot be utilised because of the deficiency of others (see Section 4.4.1). Appetites for individual amino acids can therefore be envisaged if the animal learns that one food contains too little of an amino acid for its requirements and another too much. Gous and DuPreez (1975) gave layer strain cockerels, in alternating periods of 6 or 12 hours, two diets which were individually poorly balanced but complementary in their amino acid composition. There were no significant differences in food intake or weight gain, either between the two alternating treatments or compared with controls given the two foods mixed together. Thus, the growing birds appears to have the ability to compensate for short periods on amino acid-imbalanced diets.

As shown in past research on free-choice feeding, it seems that the broilers have a "nutritional instinct" for balanced amino acid in their diet. Since lysine and

methionine are crucial amino acids in poultry nutrition, there are few reports on diet selection related to the other amino acids. However, an ability to select the appropriate diet in relation to other essential amino acids is also likely to exist. This information can be of considerable practical importance in poultry production if it is used to develop alternative strategies for better economic returns.

7.5. Conclusion

A free-choice feeding experiment was performed to test the sensitivity of growing broiler chickens to their diet on the basis of amino acid balance, especially related to lysine concentration. Over the whole period of the experiment, birds consumed some of each diet offered, but preference was shown for the moderate or high lysine diet. Growth rate reflected total lysine intake. The birds offered the choice between Ideal Protein diet and High Protein diet consumed about 2.5 times as much of the Ideal Protein diet as of the imbalanced diet. These results confirmed that the broilers have an ability to select the appropriate diet in relation to amino acid composition.

Chapter 8

GENERAL DISCUSSION

Since protein is the one of the major cost components of the poultry diet, it has a great influence on poultry production costs. Determining amino acid requirements for each phase of growth is expensive and tedious work and requirements have to be modified whenever growth characteristics change substantially. Ever since the concept of ideal protein was introduced, there has been much research to optimise protein quality for poultry by doing growth trials. In recent decades, concern for the environment has also become an important issue as waste from animal farming has increased. In this thesis, the effects of varying protein quality on energy and nitrogen metabolism, growth and diet selection in growing broiler chickens were investigated. This will contribute to a better knowledge of how growing broilers respond to differences in amino acid balance.

8.1. Effect of amino acid balance on intake and growth

The supply of balanced nutrients in animal feed is one of the most critical requirements for achieving optimal growth. Protein, especially, is the major nutrient that determines the performance of animals, assuming that energy is non-limiting. Dietary protein must be judged as a source of individual amino acids which are needed for synthesis of protein by the animal.

In the first calorimetry experiment, there was no significant effect of CP concentration ranging from 18 to 30 % of diets on food intake when lysine concentration was held constant. However, weight gain and FCE tended to be lower

as CP content increased although the difference failed to attain significance. The birds fed on the 30 % CP diet showed the lowest weight gain and FCE. The result from the growth experiment, which used diets ranging 21 to 30 % CP, also showed no significant effects of protein content on food intake and growth rate. The level of crude protein in practical diets ranges from 18 to 23 %. It is likely that the need for nitrogen to support the synthesis of non-essential amino acids will be adequately met by protein levels in this range. In this thesis, relatively high protein diets were included in the treatments and no significant effect of a high protein diet on food intake and growth was found. However, decreases in food intake with increasing dietary CP have been reported previously (*e.g.* Parsons and Baker, 1982; Fancher and Jensen, 1989; Smith and Pesti, 1998; Sklan and Plavnik, 2002). Summers *et al.* (1992) suggested that birds eat to fulfil their essential amino acid requirements. That does not appear to be the case in this experiment, as by this hypothesis, food intake should increase as the requirement increases with higher dietary CP. Theoretically, protein quality or amino acid balance should not affect bird performance as long as all amino acids meet requirement and there is no overt antagonism. In practice, however, a poorly balanced diet often results in inferior performance. This suggests that in a poor quality diet, the amino acids are in such disproportion as to impair the utilisation of the first limiting amino acid. But Boorman and Ellis (1996) reported that the limiting amino acid is used with same efficiency in good or poor quality protein diets, and that impaired growth with the latter cannot be explained on reduced utilisation of the first limiting amino acid. D'Mello (1993) investigated a lysine imbalance at 315g CP/kg diet, a high protein level for young chickens, and found no evidence of impaired utilisation of lysine, although his study didn't include attempts to maximise response. Morris *et al.* (1999) suggested that amino acid requirements will increase with increase in protein content in the diet. The second and the third

experiment, which used the diet with varying concentration of lysine as the first limiting amino acid and constant CP, showed increasing trends of food intake, growth rate and FCE as lysine concentration increased. According to Morris *et al.* (1999), simply changing CP in the diet regardless of amino acid balance, means that lysine requirement changes from 1.02 to 1.47 % of the diet, as CP increases from 18 to 26 %. Surisdiarto and Farrell (1991) showed a similar result as CP level changed from 15 to 22 %. In the varying lysine experiment, CP concentration was higher than practical range. The recommendation of lysine for 3-6 wk old broilers is 1.00 % of the diet and CP level is 20 % (NRC, 1994). The weight gain and FCE were significantly higher with the 1.2 % and 1.4 % lysine diets. It is possible that a high CP level resulted in increased lysine requirement. As mentioned in the previous chapter (see section 3.1), Morris *et al.* (1999) stated that amino acid imbalance leads to a decline in the efficiency of utilisation of the limiting amino acid. This imbalance was explained as being different from the classical antagonism between amino acid pairs such as arginine-lysine and leucine-isoleucine.

Boorman (1979) reported that diets which are imbalanced in the amino acids absorbed from the digestive tract lead to metabolic disturbances and reduced food intake. The reduction in food intake is directly proportional to the degree of amino acid deficiency or imbalance. This is thought to be due to the metabolic cost of deaminating the excess of those amino acids which cannot be utilised because of the deficiency of others. Surplus amino acids result in a disturbed free amino acid pattern in the plasma and affect appetite because of post-ingestional feed back.

Many studies also have shown that increased CP intake resulted in decreased abdominal fat (*e.g.* Bartov, 1979; Moran *et al.*, 1992; Smith and Pesti, 1998). In the

first experiment, energy retention by fat tended to decrease as CP increased and was lowest in birds fed on a 30 % CP diet. Results from the growth trial (Chapter 6) agreed with this. Although there was no significant difference in abdominal fat deposition, the birds fed on the highest CP diet showed the lowest abdominal fat deposition. When CP or amino acids are given in excess, energy is not deposited as protein, but can be used for fat synthesis after deamination. In general, it seems that control of energy intake or first limiting amino acid took priority over the control of food intake. Dietary CP content did not affect growth rate or carcass characteristics.

The effect of high ambient temperature on food utilisation, initially by reducing food intake, was also confirmed. This is due to the lesser quantity of energy required to maintain body temperature. Howlider and Rose (1987) reported that the reduction is 1.5 to 2.5% per °C increase in temperature above 20 °C in broilers. This can be seen clearly when comparison is made between the second and the third experiment performed at 20 and 30 °C respectively in this thesis.

8.2. Effect of amino acid balance on nitrogen metabolism

As the public becomes increasingly aware of the environment and the factors affecting it, the agricultural industry has been looking at ways of controlling potential pollutants such as nitrogen and phosphorus in animal wastes. Since there is a direct relationship between manure and dietary nitrogen contents, dietary protein concentration has been reduced and, at present, the content in a bird's diet is much lower than that used in the last decade (Lopez and Leeson, 1992). It is generally recognised that minimising excess dietary amino acids for chicken is desirable to

improve nitrogen utilisation and reduce nitrogen emissions into the environment. Many scientists have been trying to minimize the protein content of the diet without negatively affecting the growth or production of the bird. Although the range of the protein contents used in this thesis was higher than in practical diets, the first experiment still showed a close relationship between CP content and N excretion rate. Efficiency of N utilisation was negatively affected by increasing CP content. The results confirmed that reduction of dietary CP level had a beneficial effect in terms of reducing N excretion. In the following experiments using diets with varying lysine content, N intake and efficiency of N retention were all significantly affected by dietary lysine concentration. Protein retention was closely correlated with lysine concentration, and therefore, lysine intake.

Feeding birds in a more accurate way, as far as amino acid supply is concerned, leads to a major reduction in the excess of amino acids in the diet. This means that a lesser quantity of amino acids need to be catabolised and excreted in the urine as uric acid. It is to be expected that this leads to reduced water intake and hence to a reduced production of water in the urine. This may improve floor and litter conditions and may reduce ammonia concentration in the poultry house environment. The experiments showed that increasing CP contents or certain amino acid levels not only increased nitrogen excretion but also the dry matter of faeces produced and also water output. Reduction of water excretion may have an economical benefit in terms of costs for waste management such as manure storage or transport.

8.3. Effect of amino acid balance on energy metabolism

The feeding of unbalanced diets which leave excess nutrients to be oxidised and eliminated might increase metabolic rate. The conventional expectation has been that amino acid contents in the diet above requirement and imbalances between amino acids may increase heat increment. The results from the calorimetry experiments of this thesis suggest that the effects both of protein synthesis and of imbalanced amino acid mixtures on whole-animal heat production can be relatively small.

In the first experiment, with diets formulated to have a wide range of linearly varying protein concentrations but equal concentrations of lysine, the first-limiting amino acid, there was no detectable relationship between heat production and rate of N excretion despite a 2.5-times difference in N loss. Heat production on an imbalanced, lysine-limited, amino acid mixture was no higher than on a balanced amino acid source with the same lysine concentration. There was no indication of a stimulation of heat production by excess amino acids. In the second and the third experiment, heat production increased with rate of growth as dietary lysine content increased and the significance of the trend was reinforced when the ambient temperature was increased to 30 °C (Figure 8.1). The result suggests that heat production is strongly correlated with the first limiting amino acid intake rather than total crude protein content. This agrees with MacLeod (1997) who found that there was little indication of regulatory diet-induced thermogenesis when imbalanced amino acid mixtures or excess amino acids were fed. The results imply the over-riding quantitative importance of the cost of protein accretion relative to that of nitrogen excretion. The additional energy cost of nitrogen excretion may be counter-balanced by the reduced costs of protein

accretion. Boorman and Ellis (1996) found no adverse effect of protein quality on lysine utilisation and, indeed, detected some indication of increasing net utilisation with decreased protein quality. It was suggested that a more likely cause of impaired growth with poor quality - amino acid adequate diets, is reduced net energy resulting from an increase in gluconeogenesis. Alternately there may be increased catabolism of the limiting amino acid from muscle in order to maintain homeostasis of plasma amino acid levels. To reconsider the amino acid balance, it might be worthwhile investigating the plasma amino acid profile of birds.

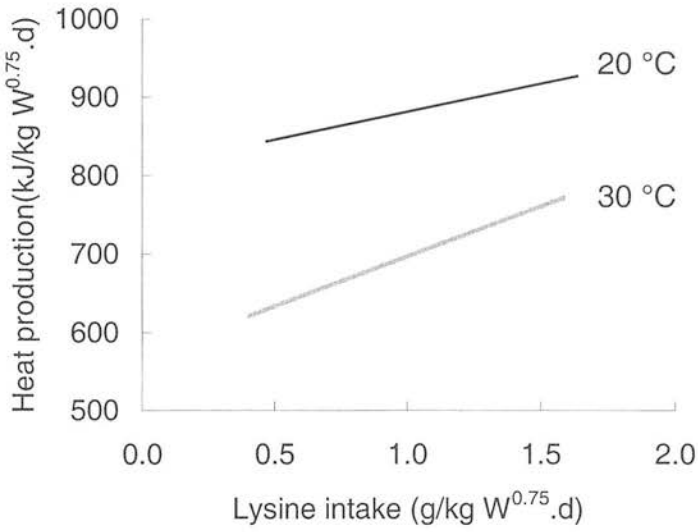


Figure 8.1 *The relationship between heat production and dietary lysine intake at high and low ambient temperature*

The relationship between lysine intake and body weight gain was similar for high and low ambient temperature, although weight gains at high temperature were somewhat lower than at low temperature (Figure 8.2). Birds at high temperature produce at a lower level because they reduce energy intake to avoid extra heat production. When birds are suffering from heat stress, they slow down metabolism by reducing growth rate or egg production. This seems to be a strategy to balance the thermoregulatory

mechanisms in order to survive severe heat stress. The production level of birds at high temperature decreased and food intake and FCE of birds responded similarly to changes in dietary lysine concentration as did birds at low ambient temperature. This similar response illustrates that the birds were able to adapt to high temperature by lowering their food intake and dietary lysine intake. It may be suggested that slow growing birds in high temperature need a lower dietary lysine: energy ratio to reach similar lysine efficiencies. This will lower dietary lysine intakes. Birds at high temperature seemed to have a lack of energy to use the extra dietary lysine for protein accretion. It can also be suggested that lysine efficiency decreases as dietary lysine intake increases. This confirms the general statement that birds use nutrients more efficiently when low levels are provided.

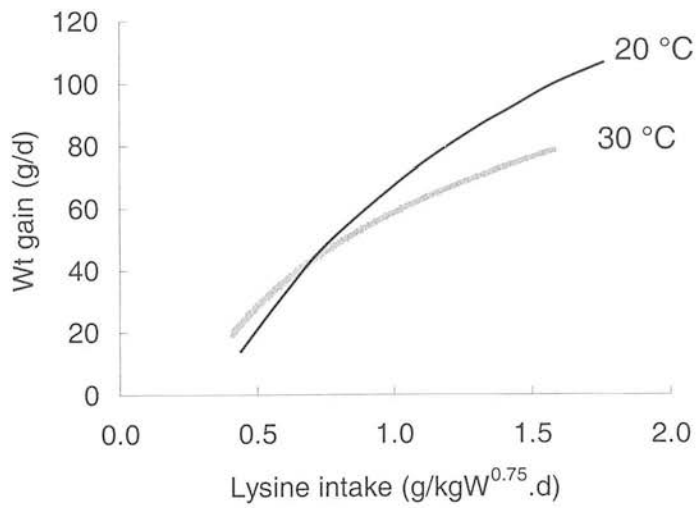


Figure 8.2 *Body weight gain response to dietary lysine intake at high and low ambient temperature*

8.4. Effect of amino acid balance on diet selection of birds

Many experiments have demonstrated that animals, including poultry, have the ability to choose an adequate diet when they are given a choice between different nutrient sources. Some scientists have shown that laying hens, growing broilers and turkeys can select adequate protein from a choice of two or three diets which are individually inadequate (*e.g.* Kishkinen, 1987; Scott and Balnave, 1989; Sinurat and Balnave, 1986; Rose and Michie, 1982).

Free choice can be used as a method for assessing the requirements and preference of the animal. Although it has been derived from scientific interest, it is possible to apply this technique to get a practical benefit in animal production. Forbes and Shariatmadari (1994) stated that major possibilities for using choice feeding to benefit the economic efficiency of poultry are : 1) its use on a small scale to find the mixture of foods which best meets the requirements of birds so that these results can be used to formulate complete single feeds to be used on a large scale, 2) its use on a commercial scale to reduce feed costs and minimize nitrogenous waste, 3) to extend the principles and practice worked out for protein to other nutrients and to discover the conditions under which diet selection can be successful when offering a choice of three or more foods.

In this study, free choice feeding experiments were performed in connection with work on the effects of amino acid balance on energy metabolism. The first experiment was carried out with diets containing the same amount of crude protein but with a range of lysine concentrations. The diets were designed so that lysine was the first-limiting amino acid. The objective was to investigate the sensitivity of the

broiler chicken to dietary amino acid balance, especially in relation to lysine concentration. As a result, birds consumed some of each diet offered, but preference was shown for the moderate or high lysine diet over the low lysine diet. Growth rate reflected total lysine intake. The second experiment also confirmed the bird's ability to select an ideal protein diet over a high-protein imbalanced diet. The birds offered the choice between the Ideal Protein diet and the High Protein diet consumed about 2.5 times as much of the Ideal Protein diet as of the imbalanced diet.

As shown in past research on free-choice feeding, it seems that the broilers have a "nutritional instinct" for balanced amino acid in their diet. Since lysine and methionine are crucial amino acids in poultry nutrition, there are few reports on diet selection related to the other amino acids. However, an ability to select the appropriate diet in relation to other essential amino acids is also likely to exist. This information can be used to find the mixture of foods which best meets the requirements of birds and to reduce feed costs and minimize nitrogenous waste.

8.5. Conclusions and suggestions for further research

Based on the results of this thesis, it may be concluded that control of first limiting amino acid or energy intake has priority in the control of food intake and growth. However, more research is needed to determine in detail which factors affect growth negatively. It should also be valuable to study effects of amino acids other than lysine to improve performance. It is clear that high ambient temperature had a large suppressing effect on food intake and performance of birds. The utilisation of protein and energy strongly interacts with ambient temperature and many of regions where the broiler industry are located have long periods of high ambient temperature that will impact negatively on growth performance. In the study of amino acid and energy requirement, therefore, the effect of ambient temperature always should be considered.

As heat production results suggested, there was no significant change in the energy cost of protein accretion between high-CP and low-CP diets. Contrary to former conventional expectations, there was no indication of a stimulation of heat production by excess amino acids. The theoretical energy cost of nitrogen excretion was relatively small and did not affect significantly the total heat production. This suggests the importance of the cost of protein accretion relative to that of nitrogen excretion. Therefore, it can be concluded that heat production is closely correlated with lysine, the first limiting amino acid, rather than with total crude protein. The HP rate and the physical activity of the birds are closely synchronous with the light cycle.

Nitrogen accretion (protein synthesis) was also strongly related to the intake of the first-limiting amino acid. Reduction in dietary CP level or balancing amino acids had a beneficial effect in terms of reducing nitrogen excretion. It may also be possible to improve efficiency of nitrogen utilisation by genetic selection. However, the extent to which birds vary in nitrogen utilisation efficiency and the extent to which this variation is heritable are not well known. Energy and amino acid requirement studies should be conducted to accomplish genetic improvement in nitrogen utilization efficiency along with growth rate in commercial broilers.

Growing broilers were able to select their diet from a choice of foods on the basis of their lysine content to satisfy their requirements. The information from choice-feeding experiments can be of considerable importance in poultry production if it is used to develop alternative strategies for better economic returns.

There has been a large amount of research on amino acid requirements and metabolism. Future progress might be best served by models based on this knowledge and descriptions of what the bird uses amino acids for. This modelling approach could enable prediction of the requirements of the birds which is difficult to detect in experiments. It is also important to do more studies about genetic factors which affect efficiency of amino acid utilisation in birds. This should be extended to determining whether there is any scope for genetically selecting birds to excrete less of the nitrogen they consume. Along with it, energy and amino acid requirement studies should be conducted frequently to take into account the genetic improvement in growth rate and N utilisation efficiency of broilers.

REFERENCES

Abebe, S. and Morris, T.R. (1990) Note on the effects of protein concentration on response dietary lysine by chicks. *British Poultry Science,* 31: 366-260.

Adams, R.L., Andrews, F.N., Rogler, J.C. and Carrick, C.W. (1962a) The protein requirement of 4-week-old chicks as affected by temperature. *Journal of Nutrition*, 77: 121-126.

Adams, R.L., Andrews, F.N., Gardiner, E.E., Fontaine, W.E. and Carrick, C.W. (1962b) The effect of environmental temperature on the growth and nutritional requirements of the chick. *Poultry Science*, 41: 558-594.

Agricultural Research Council (1981) *The Nutrient Requirements of Farm Livestock*: No.1 Poultry. Agricultural Research Council, London.

Alleman, F. and Leclercq, B. (1997) Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. *British Poultry Science,* 38: 607-610.

Almquist, H.J. (1947) Evaluation of amino acid requirement by observations on chicks. *Journal of Nutrition*, 34: 543-563.

Anderson, D.L., Hill, F.W. and Renner, R. (1958) Studies of the metabolizable and productive energy of glucose for the growing chick. *Journal of Nutrition*, 65: 561.

Anderson, J.O. and Warnick, R.E. (1967) Gross abnormalities in chicks fed amino acid deficient diets. *Poultry Science*, 46: 856-862.

Austic, R.E. (1985) Feeding poultry in hot and cold climates. In: *Stress Physiology in Livestock*. Vol.3, (Edit. M.K. Yousef) CRC Press Inc. Boca Raton, FI. USA

Baker, D.H. and Han, Y.M. (1994a) Ideal protein for broiler chicks. In: Proceedings of the Maryland Nutrition Conference, College Park, MD. pp. 269-272.

Baker, D.H. and Han, Y.M. (1994b) Ideal amino acid profile for chicks during the first three weeks post-hatching. *Poultry Science* 73: 1441-1447.

Baldini, J.T. (1961) The effect of dietary deficiency on the energy metabolism of the chick. *Poultry Science*, 40:1177-1183.

Barbour,. G., Latshaw, J.D. and Bishop, B. (1993) Lysine requirement of broiler chicks as affected by protein source and method of statistical evaluation. *British Poultry Science*, 34: 747-756.

Bartov, I. (1979) Nutritional factors affecting quantity and quality of carcass fat in chickens. *Federation Proceedings*, 38: 2627-2639.

Bartov, I. (1989) Lack of interactive effect of nicarbazin and dietary energy to protein ratio on performance and abdominal fat pad weight of boiler chicks. *Poultry Science*, 68: 1535-1539.

Bartov, I. (1996) Interrelationship between the effects of dietary factors and feed withdrawal on the content and composition of liver fat in broiler chicks. *Poultry Science*, 75: 123-133.

Bartov, I. and Plavnik, I. (1998) Moderate excess of dietary protein increases breast meat yield of broiler chicks. Poultry Science, 77: 680-688.

Blair, R., Lee, D.J.W., Fisher, C. and McCorquodale, C.C. (1976) Responses of laying hens to a low-protein diet supplemented with essential acids, L-glutamic acid, and/or intact protein. *British Poultry Science*, 17: 427-440.

Blair, R., Jacob, J.P., Ibrahim, S. and Wang, P. (1999) A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. *Journal of Applied Poultry Research*, 8: 25-47.

Blair, J.C., Harber, C.D., McNab, J.M., Mitchell, G.G. and Scougall, R.K. (1981) *Analytical Data of Poultry Feedstuffs. 1.General and Amino Acid Analyses, 1977-1980.* Occasional Publication No 1, ARC Poultry Research Centre, Roslin, Midlothian, Scotland.

Blaxter, K.L., Sir. (1989) *Energy Metabolism.* Cambridge University Press.

Bonsdorff Peterson, C. (1967) A description of an open circuit respiration apparatus for poultry. In: Energy metabolism of Farm Animals. Proceedings of the 4th Symposium (Warsaw, Poland. E.A.A.P. Publication No.12), pp.437-445.

Boomgaardt, J. & Baker, D.H. (1971) Tryptophan requirement of growing chicks as affected by dietary protein level. *Journal of Animal Science*, 33: 595-599.

Boomgaardt, J. & Baker, D.H. (1973) The lysine requirement of growing chicks fed sesame meal-gelatin diet at three protein levels. *Poultry Science*, 52: 586-591.

Boorman, K.N. (1979) Regulation of protein and amino acid intake. In: *Food intake Regulation in Poultry* (Edit. K.N. Boorman and B.M. Freeman) Edinburgh, Longman.

Boorman, K.N. (1980) Dietary constraints on nitrogen retention. In*:* *Protein Deposition in Animals*, pp. 147-166. (Edit. P.J. Buttery and D.B. Lindsay) London, Butterworths.

Boorman, K.N (1999) Protein quality and amino acid utilisation in poultry. In: *Recent Developments in Poultry Nutrition 2*, pp. 1-19. (Edit. J. Wiseman and P.C. Garnsworthy) Nottingham University Press.

Boorman, K.N. and Ellis, G.M. (1996) The maximum nutritional response to poor-quality protein and amino acid utilisation. *British Poultry Science*, 37: 145-156.

Brody, S. and Proctor, R.C. (1932) Growth and development with special reference to domestic animals : further investigations of surface area in energy metabolism. *University of Missouri Agricultural Experiment Station, Research Bulletin,* No.116.

Brouwer, E. (1957) One simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals from gaseous exchange (oxygen intake and carbonic acid output) and urine-N. *Acta physiologica et pharmacological neerlandica*, 6: 795-802.

Brouwer, E. (1965) Report of the sub-committee on constants and factors. In: Energy Metabolism of Farm Animals. (Edit. K.L. Blaxter) EAAP Publication No.11 Academic Press, London, pp. 441-443.

Buttery, P.J. and Boorman, K.N. (1976) The energetic efficiency of amino acid metabolism. In: *Protein Metabolism and Nutrition*, pp.197-204 (Edit. D.J.A. Cole) London, Butterworth.

Cahaner, A. and Leenstra, F. (1992) Effects of high temperature on growth and efficiency of male and female broilers from lines selected for high weight-gain, favorable feed conversion, and high or low fat-content. *Poultry Science*, 71: 1237-1250.

Carpenter, K.J. and De Muelenaere, H.J.H. (1965) Comparative study of performance on high protein diets of unbalanced amino acid composition. *Proceedings of Nutrition Society*, 24: 202-209.

Chung, T.K. and Baker, D.H. (1992) Ideal amino acid pattern for 10-kilogram pigs. *Journal of Animal Science*, 70: 3102-3111.

Cole, D.J.A. and Van Lunen, T.A. (1994) Ideal Amino Acid Patterns. In: *Amino acids in Farm Animal Nutrition*, pp. 99-111, (Edit. J.P.F. D'Mello) CAB International.

Chudy and Schiemann, (1971) (1983) Further experiment on choice feeding in poultry. In: *Recent Advances in Animal Nutrition in Australia.*

Cowan, P.J. and Michie, W. (1978) Environmental temperature and broiler performance: the use of diets containing increased amount of protein. *British Poultry Science*, 19:601-605.

Cumming, R.B. (1984) Choice feeding of laying birds. *Proceedings of Poultry Husbandry Research Foundation Symposium*, University of Sidney, Australia. pp. 68-71.

Cumming, R.B. (1994) Opportunities for whole grain feeding. *Proceedings of the 9th European Poultry Conference, Vol. 2.* World's Poultry Science Association. pp.219-222.

Dale, H.E., Shanklin, M.D., Johnson, H.D and Brown, W.H. (1967) Energy metabolism of the chimpanzee: a comparison of direct and indirect calorimetry, *Journal of Applied Physiology*, 22: 850-853.

Dauncey, M.J., Murgatroyd, P.R. and Cole, T.J. (1978) A human calorimeter for direct and indirect measurements of 24hr energy expenditure. *British Journal of Nutrition*, 39: 557-566.

Davis, R.H., Hassan, O.E.M. and Sykes, A.H. (1973) Energy utilization in the laying hen in relation to ambient temperature. *Journal of Agricultural Science*, 81: 173-177.

Dean, W.F. and Scott, H.M. (1965) The development of an amino acid reference diet for the early growth of chicks. Poultry Science, 44: 803-808.

De Groote, G. (1974) Utilisation of metabolisable energy. In: *Energy Requirements of Poultry* (Edit. T.R. Morris and B.M. Freenman), Poultry Science Symposium No.9, British Poultry Science Ltd., Edinburgh, pp. 113-133.

Deschepper, K. and DeGroote, G. (1995) Effect of dietary protein, essential and nonessential amino acids on the performance and carcass composition of male broiler chickens. *British Poultry Science*, 36: 229-245.

Deschutter, A. and Leeson, S. (1986) Feather growth and development. *World's Poultry Science Journal*, 42: 259-267.

D'Mello, J.P.F. and Lewis, D. (1970) Amino acid interactions in chick nutrition. 3. Interdependence in amino acid requirements. *British Poultry Science*, 11: 367-385.

D'Mello, J.P.F. (1979) Factors affecting amino acid requirements of meat birds. *Recent Advances in Animal Nutrition*, pp.1-195. (Edit. W. Haresign and D. Lewis) Butterworth, London.

D'Mello, J.P.F. (1990) Lysine utilisation by broiler chicks. In: *Proceedings of the 8th European Poultry Conference*, Barcelona, Spain. pp.302-305.

D'Mello, J.P.F. (1993) Amino acid supplementation of cereal-based diets for non-ruminants. *Animal Feed Science and Technology*, 45: 1-18.

D'Mello, J.P.F. (1994) *Amino Acids in Farm Animal Nutrition* (Edit. J.P.F. D'Mello) CAB International, Oxford.

Donaldson, W.E., Combs, G.F. and Romoser, G.L. (1956) Studies on energy levels in poultry rations. 1. The effect of calorie-protein of the ration on growth, nutrient utilization and body composition of chicks. *Poultry Science*, 35: 1100-1204.

Duke, G.E. (1986) Alimentary canal: Anatomy, regulation of feeding, and motility, In : *Avian Physiology* (Edit. P.D. Sturkie) New York, Springer-Verlag, pp.269-288.

Edmonds, M.S. and Baker, D.H. (1987) Comparative effects of individual amino acid excess when added to a corn-soybean meal diet – Effects on growth and dietary choice in the chick. *Journal of Animal Science*, 65: 699-705.

Edmonds, M.S., Parsons, C.M. and Baker, D.H. (1985) Limiting amino acids in low-protein corn-soybean meal diets fed to growing chicks. *Poultry Science*, 64: 1519-1526.

Edwards, A.C. & Campbell, R.G. (1991) Energy-protein interactions in pigs, In: *Recent Advances in Animal Nutrition-1991*, pp.3-19. (Edit. W, Haresign and D.J.A. Cole) Oxford, Butterworth-Heinemann.

Emmans, G.C. (1977) The nutrient intake of laying hens given a choice of diets in relation to their protein requirement. *British Poultry Science*, 18: 227-236.

Emmans, G.C. (1981) A model of the growth and feed intake of ad libitum fed animals particularly poultry. In: *Computers in Animal Production : proceedings of a symposium organized by the British Society of Animal Production.* (Edit. G. M. Hillyer, C. T. Whittemore and R. P. Gunn) Thames Ditton : The Society.

Emmans, G.C. (1984) An additive and linear energy scale. *Animal Production*, 38: 538-538.

Emmans, G.C. (1991) Diet selection by animals – theory and experimental design. *Proceedings of the Nutrition Society*, 50: 59-64.

Emmans, G.C. (1994) Effective energy- A concept of energy utilisation applied across species. *British Journal of Nutrition*, 71: 801-821.

Emmans, G.C. and Fisher, C. (1986) Problem in nutritional theory. In: *Nutrient Requirements of Poultry and Nutritional Research*, pp. 9-39. (Edit. C. Fisher and K.N. Boorman) Butterworth.

Farrell, D.J. (1976) The influence of protein and amino acid balance in the diet of chickens on efficiency of utilisation of dietary energy. In: *Proceedings of the 7th EAAP Symposium on the Energy Metabolism of Farm Animals*. (Edit. M. Vermorel) Vichy, France. Pp.97-100. Clermont-Ferrand, Guy de Bussac.

Farrell, D.J. and Swain, S. (1977) Effects of temperature treatments on the energy and nitrogen metabolism of fed chickens. *British Poultry Science*, 18: 735-748.

Fancher, B.I. and Jensen, L.S. (1989) Male broiler performance during the starting and growing periods as affected by dietary protein, essential amino acids and potassium levels. *Poultry Science*, 68: 1385-1395.

Ferguson, N.S., Gates, R.S., Taraba, J.L, Canton, A.H., Pescatore, A.J., Ford, M.J. and Burnham, D.J. (1998) The effect of dietary protein on growth, ammonia concentration, and litter composition in broilers. *Poultry Science*, 77: 1481-1487.

Firman, J.D. and Boling, S.D. (1998) Ideal protein in Turkeys. *Poultry Science*, 77: 105-110.

Fisher, H., Griminger, P., and Leveille, G.A (1959) Protein depletion and amino acid requirement in the growing chick. *Journal of Nutrition,* 69: 117- 123.

Fisher, H., Griminger, P., Leveille, G.A. & Shapiro, R. (1960) Quantitative aspects of lysine deficiency and amino acid imbalance. *Journal of Nutrition*, 71: 213-220.

Fisher, M.L., Leeson, S., Morrison, W.D. and Summers, J.D. (1981) Feather growth and feather composition of broiler chickens. *Canadian Journal of Animal Science*, 61: 769-773.

Forbes, J.M. (1995) *Voluntary food intake and diet selection in farm animals*. CAB International, Wallingford, UK.

Forbes, J.M. and Shariatmadari, F. (1994) Diet selection for protein by poultry. *World's Poultry Science Journal*, 50: 7-24.

Funk, E.M. (1932) Can the chick balance its ration? *Poultry Science*, 11: 94-97.

Geraert, P.A., Guillaumin, S. and Leclercq, B. (1993) Are genetically lean broilers more resistance to hot climate? *British Poultry Science*, 34: 643-653.

Gleeves, E.W., Tonkinson, L.V., Wolf, J.D. Harman, C.L., Thayer, R.H. and Morrison, R.D. (1968) The action and interaction of physiological food intake regulators in the laying hen. *Poultry Science*, 47: 38-47.

Gonder, E. and Barnes, H.J. (1987) Focal ulcerative dermatitis ("breast buttons") in marketed turkeys. *Avian Diseases*, 31(1): 52-58.

Gous, R.M. and Dupreez, J.J. (1975) The sequential feeding of growing chickens. *British Journal of Nutrition*, 34:113-117.

Graham, W.R. (1932) Can we learn anything from free-choice of feeds as expressed by chicks? *Poultry Science*, 11: 365-366.

Grau, C.R. (1948) Effect of protein level on the lysine requirement of the chick. *Journal of Nutrition*, 36: 99-108.

Greene, J.A., McCracken, R.M. and Evans, R.T. (1985) A contact dermatitis of broilers - clinical and pathological findings. *Avian Pathology*, 14: 23-38.

Guillaume, J. and Summer, J.D. (1970) Maintenance energy requirement of the rooster and influence of plane of nutrition on metabolizable energy. *Canadian Journal of Animal Science*, 50: 363-369.

Hakansson, J., Eriksson, S. and Svensson, S.A. (1978) The influence of feed energy level on feed consumption, growth and development of different organs of chicks. *Report No. 57 and 59. Swedish University of Agricultural Sciences*, Uppsala.

Halnan, E.T. (1951) The nutritive (energy) value of poultry food. *Proceedings of the 9th World's Poultry Congress,* Paris, France, pp.36.

Han, Y.M. and Baker, D.H. (1991) Lysine requirement of fast-growing and slow-growing broiler chicks. *Poultry Science*, 70: 2180-2114.

Han, Y.M. and Baker, D.H. (1993) Effects of sex, heat-stress, body-weight, and genetic strain on the dietary lysine requirement of broiler chicks. *Poultry Science*, 72: 701-708.

Han, Y.M. and Baker, D.H. (1994) Digestible lysine requirement of male and female broiler chicks during the period 3 to 6 weeks posthatching. *Poultry Science*, 73: 1739-1745.

Han, Y.M., Suzuki, H. and Baker, D.H. (1992) Amino acid fortification of a low-protein corn and soybean-meal diet for chicks. *Poultry Science*, 71:1168-1178.

Harper, A.E. and Rogers, Q.R. (1965) Amino acid imbalance. *Proceedings of the Nutrition Society*, 24:173-190.

Harper, A.E., Benevenga, N.J. & Wohlheuter, R.M. (1970) Effect of ingesting disproportionate amounts of amino acids. *Physiological Revie*ws, 50: 428-558.

Hill, F.M. and Anderson, D.L. (1958) Comparison of metabolizable energy and productive energy determinations with growing chicks. *Journal of Nutrition*, 64: 587-603.

Hill, F.W. and Dansky, L.M. (1954) Studies of the energy requirements of chickens. 1. The effect of dietary energy level on growth and feed consumption. *Poultry Science*, 33: 112-119.

Hoffmann, L. and Schiemann, R. (1971) Verdaulichkeit und energiekennzahlen von futterstoffen beim huhn. *Archiv Tierernahrung*, 21: 65-81.

Holcombe, D.J., Roland, D.A. and Harms, R.H. (1976) The ability of hens to regulate protein intake when offered a choice of diets containing different levels of protein. *Poultry Science*, 55: 1731-1737.

Holsheimer, J.P. (1981) The protein and amino acid requirements of broiler between 5 and 6 weeks. 2 Feeding diets supplemented with essential and non-essential amino acids. *Archiv Fur Geflugelkunde*, 45: 151-157.

Holsheimer, J.P. and Janssen, W.M.M.A. (1991) Limiting amino acid in low protein maize-soybean meal diets fed to broiler chicks from 3 to 7 weeks of age. *British Poultry Science*, 32: 151-158.

Holsheimer, J.P. and Veerkamp, C.H. (1992) Effect of dietary energy, protein, and lysine content on performance and yield of two strains of male broiler chicks. *Poultry Science*, 71: 872-879.

Howlider, M.A.R. and Rose, S.P. (1987) Temperature and the growth of broilers. *World's Poultry Science Journal,* 43:228-237.

Hughes, B.O. (1984) The principles underlying choice feeding behaviour in fowls-with special reference to production experiments. *World's Poultry Science Journal,* 40: 141-150.

Hughes, B.O. and Wood-Gush, D.G.M. (1971a) A specific appetite for calcium in domestic chicken. A*nimal Behavior*, 19: 490-499.

Hughes, B.O. and Wood-Gush, D.G.M. (1971b) Investigations into specific appetites for sodium and thiamine in domestic fowl. *Physiology and Behavior*, 6: 331-339.

Hurwitz, S., Plavnik, I., Bengal, I., Talpaz, H. and Bartov, I. (1983) The amino acid requirements of growing turkeys. 2. Experimental validation of model-calculated requirements for sulfur amino acids and lysine. *Poultry Science*, 62: 2387-2393.

Ishibashi, T. (1990) Amino acid requirement of poultry. *Japanese Poultry Science*, 27: 1-15.

Jackson, S., Summers, J.D. and Leeson, S. (1982) Effect of dietary protein and energy on broiler carcass and efficiency of nutrient utilization. *Poultry Science*, 61: 2224-2231.

James, E.W., Jr. and Wheeler, R.S. (1949) Relationship of dietary protein content to water intake, water elimination and amount of cloacal excreta produced by growing chickens. *Poultry Science*, 28:465- 467.

Jequier, E. and Schutz, Y. (1983) Long term measurements of energy expenditure in humans using a respiratory chamber. *American Journal of Clinical Nutrition*, 38: 989-998.

Katz, R.S. and Baker, D.H. (1975) Methionine toxicity in the chick: nutritional and metabolic implications. *Journal of Nutrition*, 105: 1168-1175.

Kaufmann, L.W., Collier, G. and Squibb, R.L. (1978) Selection of adequate protein-carbohydrate ratio by domestic chicks. *Physiology and Behavior*, 20: 339-344.

Kempster, H.L. (1916) Food selection by laying hens. *Journal of the American Association of Institution and Investigators in Poultry Husbandry*, 3: 26-28.

Kerr, B.J., Kidd, M.T., McWard, G.W. and Quarles, C.L. (1999) Interactive effects of lysine and threonine on live performance and breast yield in male broilers. *Journal of Applied Poultry Research*, 8: 391-399.

Keshavarz, K. (1991) The effect of calcium-sulfate (Gypsum) in combination with different sources and forms of calcium-carbonate on acid-base balance and eggshell quality. *Poultry Science*, 70 (8): 1723-1731.

Keshavarz, K and Jackson, M.E. (1992) Performance of growing broiler pullets and laying hens fed low-protein amino acid-supplemented diets. Poultry Science, 71: 905-918.

Khalil, A.A., Thomas, O.P. and Combs, G.F. (1968). Influence of body composition, methionine deficiency or toxicity and ambient temperature on feed intake in the chick. *Journal of Nutrition,* 96: 337- 341.

Kidd, M.T. and Fancher, B.I. (2001) Lysine needs of starting chicks and subsequent effects during the growth period. *Journal of Applied Poultry Research*, 10: 385-393.

Kiiskinen, T. (1987) Influence of choice feeding on the performance of growing pullets and laying hens. *Annales Agriculturae Fenniae*, 26: 131- 144.

Klandorf, H., Sharp, P.J. and MacLeod, M.G. (1981) The relationship between heat production and concentration of plasma thyroid hormones in the domestic hen. *General and Comparative Endocrinology*, 45:513-520.

Klasing, K.C. (1998) Energy. In: *Comparative Avian Nutrition*, Wallingford, CAB International. pp. 210-233.

Kleiber, M. (1932) Body size and metabolism. *Hilgardia*, 6: 315-353.

Kleiber, M. and Dougherty, J.E. (1934) The influence of environmental temperature on utilization of food energy in baby chicks. *Journal of General Physiology*, 17: 701-726.

Kubena, L.F., Lott, B.D., Deaton, J.W. Reece, F.N. and May, J.D. (1972) Body composition of chicks as influenced by environmental temperature and selected dietary factors. *Poultry Science*, 51: 517-522.

Kyriazakis, I., Emmans, G.C. and Whittemore, C.T. (1990) Diet selection in pigs-choice made by growing pigs given foods of different protein concentrations. *Animal Production*, 51: 189-199.

Lerlercq, B. (1998) Specific effects of lysine on broiler production: Comparison with threonine and valine. *Poultry Science*, 77:118-123.

Leeson, S. and Summers, J.D. (1978) Dietary self selection by turkeys. *Poultry Science*, 57: 1579-1585.

Leeson, S. and Summers, J.D. (1991) Broiler diet specifications. In: *Commercial Poultry Nutrition*, p. 151. University Books, Guelph, ON, Canada.

Leeson, S., Caston, L. and Summers, J.D. (1996) Broiler response to diet energy. *Poultry Science*, 75: 529-535.

Leeson, S., Diaz, G. and Summers, J.D. (1995) Ascites. In: *Poultry Metabolic Disorders and Mycotoxins*, pp. 43-44. University Books, Guelph, ON, Canada.

Lehmann, D, Pack, M and Jeroch, H. (1996) Responses of growing and finishing turkey toms to dietary lysine. *Poultry Science*, 75: 711-718.

Lipstein, B., Bornstein, S. and Bartov, I. (1975) The replacement of some of the soybean meal by the first-limiting amino acids in practical broiler diets 3. Effect of protein concentrations and amino acid supplementations in broiler finisher diet on fat deposition in the carcase. *British Poultry Science*, 16: 627-635.

Livesey, G. (1984) The energy equivalents of ATP and the energy values of food protein and fats. *British Journal of Nutrition*, 51: 15-28.

Lopez, G. and Leeson, S. (1995) Response of broiler breeders to low-protein diets. 1. Adult breeder performance. *Poultry Science*, 74: 685-695.

Lundy, H., MacLeod, M.G and Jewitt, T.R. (1978) An automated multi-calorimeter system: experiments on laying hens. *British Poultry Science*, 19: 173-186.

Lunven, P., Le Clement de St Marcq, C., Carnovale, E. and Fratoni, A.(1973) Amino acid composition of the hen's egg. *British Journal of Nutrition,* 30: 189-194.

Mack, S., Bercovici, D., De Groote, G., Leclercq, B., Lippens, M., Pack, M., Schutte, J.B. and Van Cauwenberghe, S. (1999) Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. *British Poultry Science*, 40: 257-265.

MacLeod, M.G. (1990) Energy and nitrogen intake, expenditure and retention at 20 degree in growing fowl given diets with wide range of energy and protein contents. *British Journal of Nutrition*, 64: 625-637.

MacLeod, M.G. (1991) Effects of feeding by crop intubation on energy metabolism and physical activity in domestic cockerels. *British Poultry Science*, 32: 1089-1095.

MacLeod, M.G. (1992) Energy and nitrogen intake, expenditure and retention at 32 degree in growing fowl given diets with wide range of energy and protein contents. *British Journal of Nutrition*, 67: 195-206.

MacLeod, M.G. (1994) Dietary energy utilization: prediction by computer simulation of energy metabolism. In: *Proceedings of the 13th Symposium on Energy Metabolism of Farm Animals*. (Edit. J. Aguilera) September 18-24, 1994, Spain, pp.237-240

MacLeod, M.G. (1997) Effects of amino acid balance and energy: protein ratio on energy and nitrogen metabolism in male broiler chickens. *British Poultry Science*, 38: 405-411.

MacLeod, M.G. & Boyd, A. (1994) Imbalanced amino acid mixtures, low and high protein:energy ratios: their effects on energy metabolism, in*:* *Proceedings 9th European Poultry Conference,* Glasgow UK, pp. 531-532 (World's Poultry Science Association, UK Branch).

MacLeod, M.G., Jewitt, T.R. and Anderson, J.E.M. (1988) Energy expenditure and physical activity in domestic fowl kept on standard and interrupted lighting patterns. *British Poultry Science*, 29:231-244.

MacLeod, M.G., Lundy, H. and Jewitt, T.R. (1985) Heat production by the mature male turkey (*Meleagris gallopavo*): preliminary measurements in an automated, indirect, open-circuit multi-calorimeter system. *British Poultry Science*, 26: 325-33.

MacLeod, M.G., Tulletts, S.G. and Jewitt, T.R. (1980) Circadian variation in the metabolic rate of growing chickens and laying hens of a broiler strain. *British Poultry Science*, 21: 155-159.

MacLeod, M.G., Jewitt, T.R., White, J., Verbrugge, M. and Mitchell, M.A. (1982) The contribution of locomotor activity to energy expenditure in the domestic fowl. In: *Energy Metabolism of Farm Animals, Proceedings of the 9th EAAP Symposium.* (Edit. A. Ekern and E. Sundstol) Department of Animal Nutrition, Agricultural University of Norway, Aas, pp.297-300.

March, B.E. and Biely, J. (1972) The effect of energy supplied from the diet and from environment heat on the response of chicks to different levels of dietary lysine. *Poultry Science*, 51: 665-668.

Marks, H.L. and Pesti, G.M. (1984) The roles of protein level and diet form in water consumption and abdominal fat pad deposition of broilers. *Poultry Science*, 63: 1617-1625.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D and Morgan, C.A. (1995) *Animal Nutrition*, 5th Edition. Longman Scientific & Technical, Harlow, Essex, UK.

McLean, J.A. and Tobin, G. (1987) *Animal and human calorimetry*. Cambridge University Press.

McNab, J.M. (1994) Amino acid digestibility and availability studies with poultry. In: *Amino Acid in Farm Animal Nutrition*, pp. 185-203. (Edit. J.P.F. D'Mello) CAB International.

McNab, J.M. and Blair, J.C. (1988) Modified assay for true and apparent metabolisable energy based on tube-feeding. *British Poultry Science*, 29: 697-707.

McNaughton, J.L., May, J.D., Reece, F.N. and Deaton, J.W. (1978) Lysine requirement of broilers influenced by environmental temperatures. *Poultry Science*, 57: 57-64.

Meluzzi, A., Sirri, F., Tallarico, N. and Franchini, A. (2001) Nitrogen retention and performance of brown laying hens on diets with different protein content and constant concentration of amino acid and energy. *British Poultry Science*, 42: 213-217.

Mendonca , C.X. and Jensen, L.S. (1989) Influence of protein concentration on the sulphur-containing amino acid requirement of broiler chickens. *British Poultry Science*, 30: 889-898.

Mickelberry, W.C., Rogler, J.C. and Stadelman, W.J. (1966) The influence of dietary fat and environmental temperature upon chick growth and carcass composition. *Poultry Science*, 45: 313-321.

Millward, D.J., Garlick, P.J. and Reeds, P.J. (1976) The energy cost of growth. *Proceedings of Nutrition Society*, 35: 339-349.

Misson, B.H. (1974) An open-circuit respirometer for metabolic studies on the domestic fowl: establishment of standard operating conditions. *British Poultry Science*, 15: 287-297.

Mitchell, H.H. (1962) *Comparative Nutrition of Man and Domestic Animals*. New York, Academic Press.

Moran, E.T., Jr. (1994) Responses of broiler strains differing in body-fat to inadequate methionine ; live performance and processing yields. *Poultry Science*, 73: 1116-1126.

Moran, E.T., Jr., Bushong, R.D. and Bilgili, S.F. (1992) Reducing dietary crude protein for broilers while satisfying amino acid requirements by least-cost formulation: live performance, litter composition, and yield of fast-food carcass cuts at six weeks. *Poultry Science*, 71: 1687-1694.

Morris, T.R. (1968) The effect of dietary energy level on the voluntary calorie intake of laying birds. *British Poultry Science*, 9:285-295.

Morris, T.R. and Abebe, S. (1990) Effects of arginine and protein on chicks response to dietary lysine. *British Poultry Science*, 31: 261-266.

Morris, T.R., Gous, R.M. and Fisher, C. (1999) An analysis of the hypothesis that amino aicd requirements for chicks should be stated as a proportion of dietary protein. *World's Poultry Science Journal*, 55: 7-22.

Morris, T.R., Al-Azzawi, K., Gous, R.M. & Simpson, G.L. (1987) Effects of protein concentration on responses to dietary lysine by chicks. *British Poultry Science*, 28: 185-195.

Murphy, M.E. and King, J.R. (1989) Sparrows discriminate between diets differing in value or lysine concentrations. *Physiology and Behavior*, 45: 423-430.

Musharaf, N.A. and Latshaw, J.D. (1999) Heat increment as affected by protein and amino acid nutrition. *World's Poultry Science Journal*, 55: 233-240

National Research Council (1984) *Nutrient Requirements of Poultry*. National Academic Press, Washington, D.C.

National Research Council (1994) *Nutrient Requirements of Poultry*. 9th Revised Edition. National Academic Press, Washington, D.C.

Nelson, T.S., Young, R.J., Bradfield, R.B., Anderson, J.B., Norris, LC., Hill, F.W. and Scott, M.L. (1960) Studies on the sulfur amino acid requirements of the chick. *Poultry Science*, 39: 308-314.

Netke, S.P., Scott, H.M., & Allee, G.L. (1969) Effect of excess amino acid on the utilisation of the first limiting amino acid in chick diets. *Journal of Nutrition*, 99: 75-81.

Newman, R.K. and Sands, D.C. (1993) Dietary selection for lysine by the chick. *Physiology and Behavior*, 31: 13-19.

Nitsan, Z., Dvorin, A. and Nir, I. (1981) Composition and amino acid content of carcass, skin and feathers of the growing gosling. *British Poultry Science*, 22: 79-84.

Noble, D.O., Picard, M.L., Dunnington, E.A., Uzu, G., Larsen, A.S. and Siegel, P.B. (1993) Food intake adjustments of chicks – Short term reactions of genetic stocks to deficiencies in lysine, methionine or tryptophan. *British Poultry Science*, 34: 725-735.

Okumura, J. and Mori, S. (1979) Effects of deficiencies of single amino acid on nitrogen and energy utilisation in chicks. *British Poultry Science*, 20: 421-429.

Olson, D.W., Sunde, M.L and Bird, H.R. (1972) The effect of temperature on metabolizable energy determination and utilization by the growing chick. *Poultry Science*, 51: 1915-1922.

Parr, J.F. and Summers, J.D. (1991) The effect of minimizing amino acid excesses in broiler diets. *Poultry Science*, 70: 1540-1549.

Parsons, C.M. and Baker, D.H.(1982) Effect of dietary protein level and monensin on performance of chicks. *Poultry Science*, 61: 2083-2088.

Patrick, H. and Ferrise, A. (1961) The water intake of broilers. *Poultry Science*, 41: 1363-1367.

Payne, C.G. (1967) Layer response to energy. In: *Environmental Control of Poultry Production*, pp.40-54. (Edit. Carter, T.C.) Longmans, London, UK.

Picard, M.L., Uzu, G., Dunnington, E.A. and Siegel, P.B. (1993) Food intake adjustments of chicks- short-term reactions to deficiencies in lysine, methionine and tryptophan. *British Poultry Science*, 34 (4): 737-746

Pinchasov, Y., Mendonca, C.X. and Jensen, L.S. (1990) Broiler chick response to low protein diets supplemented with synthetic amino acids. *Poultry Science*, 69: 1950-1955.

Pirgozliev, V. and Rose, S.P. (1999) Net energy system for poultry feeds: a quantitative review. *World's Poultry Science Journal*, 55: 23-36.

Reeds, P.J. and Fuller, M.F. (1983) Nutrient intake and protein turnover. *Proceedings of the Nutrition Society*, 42: 463-471.

Reeds, P.J., Haggarty, P., Wahle, K.W.J and Fletcher, J.M. (1982) Tissue and whole-body protein synthesis in immature zucker rats and their relationship to protein deposition. *Biochemical Journal*, 204: 393-398.

Rhone-Poulenc Animal Nutrition (1993) Rhodimet Nutrition Guide, *Feed ingredients formulation in digestible amino acids*, 2nd edition, RPAN

Robbins, K.R. (1987) Threonine requirement of the broiler chick as affected by protein level and source. *Poultry Science*, 66: 1531-1534.

Rodgers, W. and Rozin, P. (1966) Novel food preferences in thiamine-deficient rats. *Journal of Physiology and Psychology*, 61:1-4.

Romjin, C. and Lokhorst, W. (1961) Some aspects of energy metabolism in birds. In: *2nd Symposium on Energy Metabolism*, pp. 49-59. Wageningen, E.A.A.P. Publication No.10.

Romjin, C. and Lokhorst, W. (1966) Heat regulation and energy metabolism in the domestic fowl. In: *Physiology of Domestic Fowl*. (Edit. C. Horton-Smith and E.C. Amoroso) pp.211-227. Edinburgh, Oliver and Boyd.

Rose, S.P. and Kyriazakis, I. (1991) Diet selection of pigs and poultry. *Proceedings of Nutritional Society*. 50: 87-98.

Rose, S.P. and Lambie, I.T.M. (1986) Comparison of a choice-feeding regimen for broilers under continuous and intermittent lighting programmes. *Proceedings of the 7th European Poultry Conference, Paris, World's Poultry Science Association*, pp 903-906.

Rose, S.P. and Michie, W. (1982) The food intakes and growth of choice-fed turkeys offered balancer mixtures of different compositions. *British Poultry Science*, 23: 547-554.

Roth, F.X., Steinruck, U. and Kirchgessner, M. (1990) Selective feed intake of layer during methionine deficiency. *Archiv fur Geflugelkunde*, 54: 204-211.

Rubner, M. (1883) Über die einflussder korpergrosse auf stoff und kraftvechesel. *Zeitschrift für Biologie*, 19: 535-562.

Rugg, W.C. (1925) Feeding experiments, free choice of feeds. *Department of Agriculture Bulletin, Victoria, Australia,* 54: 36-46.

Savory C.J. (1986) Influence of ambient temperature on feeding activity parameters and digestive function in domestic fowls. *Physiology and Behavior*, 38:353-357.

Schulz, A.R. (1975) Computer-based method for calculation of the available energy of proteins. *Journal of Nutrition*, 105: 200-207.

Schutte, J.B. (1987) Utilization of synthetic amino acids in poultry. In: *6th European Symposium on Poultry Nutrition*, pp. 11-12 (Koningslutter, Germany, World's Poultry Science Association).

Scott, T.A. and Balnave, D. (1989) Responses of sexually maturing pullets to self-selection feeding under different temperature and lighting regimens. *British Poultry Science*, 30: 135-150.

Scott, M.L., Neshein, M.L. and Young, R.L. (1969) *Nutrition of the Chicken*. (Edit. M.L. Scott and Assoc.) Ithaca, New York. pp.49.

Shariatmadari, F. and Forbes, J.M. (1993) Growth and food intake responses to diets of different protein contents and a choice between diets containing 2 concentrations of protein in broiler and layer strains of chicken. *British Poultry Science*, 34: 959-970.

Si, J., Fritts, C.A., Burnham, D.J. and Waldroup, P.W. (2001) Relationship of dietary lysine level to the concentration of all essential amino acids in broiler diets. *Poultry Science*, 80: 1472-1479.

Sibbald, I.R. (1976) A bioassay for the true metabolizable energy in feedstuffs. *Poultry Science*, 55: 303-308.

Siegel, P.B., Picard, M., Nir, I., Dunnington, E.A., Willemsen, M.H.A. and Williams, P.E.V. (1997) Response of meat-type chickens to choice feeding of diets differing in protein and energy from hatch to market weight. *Poultry Science*, 76:1183-1192.

Sinurat, A.P. and Balnave, D. (1985) Effect of dietary amino acids and metabolisable energy on the performance of broilers kept at high temperature. *British Poultry Science*. 26: 117-128.

Sinurat, A.P. and Balnave, D. (1986) Free-choice feeding of broiler at high temperatures. *British Poultry Science*, 27: 577-584.

Sklan, D. and Plavnik, I. (2002) Interactions between dietary crude protein and essential amino acid intake on performance in broilers. *British Poultry Science*, 43: 442-449.

Smith, E.R. and Pesti, G.M. (1998) Influence of broiler strain cross and dietary protein on the performance of broilers. *Poultry Science*, 77: 276-281.

Steinruck, U., Kirchgessner, M. and Roth, F.X. (1990a) Selective methionie intake of broilers by changing the position of the diets. *Archiv fur Geflugelkunde*, 54:245-250.

Steinruck, U., Roth, F.X. and Kirchgessner, M. (1990b) Selective feed intake of broilers during methionie deficiency. *Archiv fur Geflugelkunde*, 54: 173-183.

Stephens, D.W. and Krebs, J.R. (1986) *Foraging Theory*. Princeton University Press, Princeton. pp. 247

Stilborn, H.L. and Waldroup, P.W. (1989) Utilization of lo-protein grower diets for broiler chickens. *Poultry Science*, 68 (Suppl.1): 142.

Sugahara, K. and Kubo, T. (1992) Effects of dietary tryptophan level and food intake on energy utilization by male growing chicks. *Asian-Australian Journal of Animal Sciences*, 5: 647-651.

Sugahara, K, Kubo, T. and Tasaki, I. (1985) Involvement of feed intake in the decreased energy retention in chicks fed an arginine-deficient diet. Japanese Poultry Science, 22: 45-54.

Summers, J.D. (1993) Reducing nitrogen excretion of the laying hen by feeding lower crude protein diet. *Poultry Science*, 72: 1473-1478.

Summers, J.D. and Leeson, S. (1979) Diet presentation and feeding, In: *Food Intake Regulation in Poultry* (Edit. K.N. Boorman and B.N. Freeman) Edinburgh, Longman. pp. 445-469.

Summers, J.D. and Leeson, S. (1985) Broiler carcass composition as affected by amino acid supplementation. *Canadian Journal of Animal Science*, 65: 717-723.

Summers, J.D., Spratt, D. and Atkinson, J.L. (1992) Broiler weight gain and carcass composition when fed diets varying in amino acid balance, dietary energy and protein level. *Poultry Science*, 71: 263-273.

Surisdiarto and Farrell, D.J. (1991) The relationship between dietary crude protein and dietary lysine requirement by broiler chicks on diets with and without the "ideal" amino acid balance. *Poultry Science*, 70: 830-836.

Swain, S. and Farrell, D.J. (1975) Effects of different temperature regimens on body composition and carry-over effects on energy metabolism of growing chickens. *Poultry Science*, 54: 513-520.

Sykes, A.H. (1977) Nutrition-environment interaction in poultry, in: *Nutrition and Climatic Environment* (Edit. W. Haresign, H. Swan and D. Lewis) London, Butterworth, pp. 17-29.

Tamminga, S. (1992) Gaseous Pollutants Produced by Farm Animal Enterprises. In: *Farm Animals and the environment* (Edit. C. Phillips and D. Piggins), CAB International, pp. 345-357.

Tasaki, I., Okumura, J., Ishimoto, Y. and Katsu, K. (1976) Effects of lysine deficiency on energy utilisation in growing chicks. In: *Proceedings of the 14th World's Poultry Congress*, Madrid. pp.1133-1136.

Tasaki, I., Sugahara, K. and Okumura, J. (1976) Effect of amino acid deficiency on energy and protein utilisation in growing broiler chicks. In: *Proceedings of the 7th EAAP Symposium on the Energy Metabolism of Farm Animals*, Vichy, France. (Edit. M. Vermorel) pp. 101-104. Clermont-Ferrand, Guy de Bussac.

Temim, S., Chagneau, A.M., Guilaumin, S., Michel, J., Peresson, R., Geraert, P.A. and Tesseraud, S. (1999) Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. *Reproduction, Nutrition, Development*, 39: 145-146.

Tesseraud, S., Peresson, R., Lopes, J. and Chagneau, A.M. (1996) Dietary lysine deficiency greatly affects muscle and liver protein turnover in growing chickens. *British Journal of Nutrition*, 75: 853-865.

Thomas, O.P and Combs, G.F. (1967) Relationship between serum protein level and body-composition in the chick. *Journal of Nutrition*, 91:468-472.

Tobin, G., Boorman, K.N. & Lewis, D. (1973) Food utilisation, growth and amino acid utilisation with increasing severity of amino acid imbalance. In: *4th European Poultry Conference, London*, pp. 307-315.

Twining, P.V.,Jr., Thomas, O.P., Bossard, E.H. and Nicholson, J.L. (1974) The effect of amino acid and protein level on body composition of 8 1/2 week broilers. In: *Proceedings of the Maryland Nutrition Conference*, pp. 89-95.

Uzu, G. (1982) Limit of reduction of the protein level in broiler feeds. *Poultry Science*, 61: 1557-1558.

Uzu, G., Picard, M., Dunnington, E.A. and Siegel, P.B. (1993) Feed intake adjustments by hens to feeding regimens in which dietary methionine is varied. *Poultry Science*, 72: 1656-1662.

Vermorel, M., Bouvier, J.C., Bonnet, Y. and Fanuconneau, G. (1973) Construction et foncionment de 2 chambres respiratires du type "circuit ouvert" pour jeunes bovines. *Annales de Biologie Animale Biochimie Biophysique*, 13: 659-681.

Verstegen, M.W.A., van der Hel, W., Koppe, R. and van Es, A.J.H. (1971) An indirect calorimeter for the measurement of the heat production of large groups of animals kept together. *Mededelinge Landbouwhogeschool Wageningen*, 71: 1-13.

Vo, K.V. and Fanguy, R.C. (1978) Effects of early temperature stress on performance of commercial broilers. *Poultry Science*, 61: 1562- 1562.

Waibel, P.E. and MacLeod, M.G. (1995) Effects of cycling temperature on growth, energy metabolism and nutrient retention of individual male turkeys. *British Poultry Science*, 36: 39-49.

Wallis, I.R. (1999) Dietary supplements of methionine increase breast meat yield and decrease abdominal fat in growing broiler chickens. *Australian Journal of Experimental Agriculture*, 39: 131-141.

Wang, T.C. and Fuller, M.F. (1989) The optimum dietary amino acid pattern for growing pigs. 1. Experiment by amino acid deletion. *British Journal of Nutrition*, 62: 77-89.

Wethli, E., Morris, T.R. and Shresta, T.P. (1975) The effect of feeding high levels of low-quality proteins to growing chickens. *British Journal of Nutrition*, 34: 363-373.

Wheeler, R.S and James, E.C. (1950) The problem of wet poultry house litter : Influence of total dietary protein and soybean meal content on water intake and urinary and fecal water elimination in growing chickens. *Poultry Science*, 29: 496-500.

Wheeler, K.B. and Latshaw, T.D. (1981) Sulphur amino acid requirements and interactions in broilers during two growth periods. *Poultry Science*, 60: 228-236

Wiernusz, C.J. and Teeter, R.G. (1993) Feeding effects on broiler thermo-balance during thermoneutral and high ambient temperature exposure. *Poultry Science*, 72: 1917-1924.

Young, B.A., Kerrigan, B. and Christopherson, R.J. (1975) A versatile respiratory pattern analyser for studies of energy metabolism of livestock. *Canadian Journal of Animal Science*, 55: 17-22.

Zhou, W.T. and Yamamoto, S. (1997) Effects of environmental temperature and heat production due to food intake on abdominal temperature, shank skin temperature and respiration rate of broilers. *British Poultry Science*, 38: 107-114.

APPENDIX

DIURNAL VARIATION IN HEAT PRODUCTION RELATED TO DIET AND ACTIVITY IN GROWING BROILERS

Introduction

Physical activities require large amounts of energy for muscle contraction. It is common for these activities to expend five to ten times basal metabolic rate (Klasing, 1998). There have been some reports on the heat production of poultry and the factors affecting it have been studied widely, but there is limited information on the heat production associated with physical activity. In most studies, heat production (HP) is determined as an average over a relatively long period, such as $\geq$ 1d. HP, however, varies considerably within a day. Variation within a day is related mostly to feeding strategy and to photoperiodicity (Blaxter, 1989). Environmental temperature also affects the level and variation of heat produced during a day. In this thesis, the diurnal variation in HP and physical activity were measured throughout the calorimetry experiments.

Method

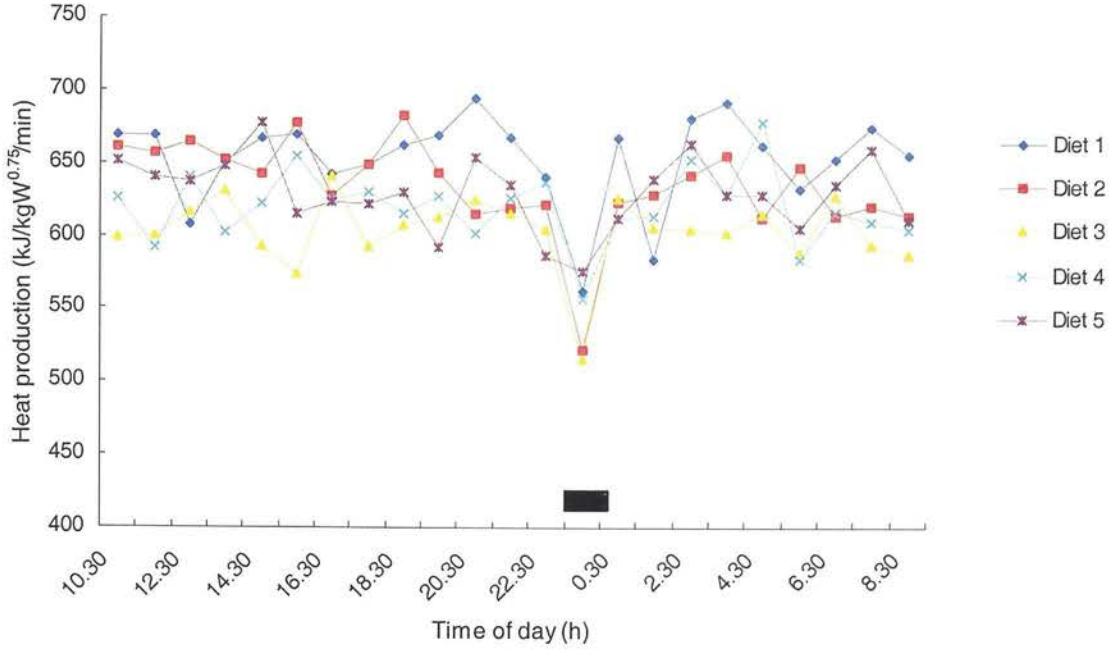
Three-week-old male broilers were used. Detailed procedures were described in the previous chapters. During the measurement period, birds received diets *ad libitum* for 3 d and were fasted for 2 d. Fasted HP was measured during the second day of fasting. The birds had free access to water throughout the experimental period. The lighting pattern was 23L: 1D, which is commonly used in commercial broiler husbandry. The 1 h of dark period started at 00.00 h and ended at 01.00 h. HP was measured hourly from 10.30 to 08.30h. From 09.00 to 10.30 h, food intake and body weight were recorded and the diet was given. Physical activity was measured simultaneously with HP by means of a Doppler-radar meter situated on top of each chamber. With this system, quantitative estimates of the activity of the birds were recorded in the same intervals as for total HP.

Result and discussion

Diurnal variations in HP in three calorimetry experiments are shown in the Figures 1, 2 and 3. HP on during both fed and fasted days decreased rapidly to a minimum during the 1-h dark period. Average HP during the dark period was approximately

30-35% lower than during the light period. This indicates that about 65-70% of the extra HP in the light period compared with the dark period was mostly related to physical activity. Metabolic rate decreased rapidly from that of light phase to that of the dark phase when the lights switched off. When the lights were switched on, HP increased equally rapidly and slightly overshot before returning to the light phase rate. The decrease during dark period of fasted birds was less obvious compared to when they were fed. MacLeod *et al.* (1985) reported that HP decreased during the dark period by about 20% in fed and about 15% in fasted turkeys. Fasted HP still showed a tendency to decrease linearly on the second day of fasting. It seems that the birds were still approaching to the basal metabolism. However, fasting could not be continued since birds would get stressed from starving. Variation was greater when birds were fed.

HP varied considerably during a day, especially due to variation in physical activity (Figure 4). Both HP and activity decreased rapidly and were minimal during dark period. HP and physical activity were highly correlated ($p<0.001$) as shown in Figure 5. HP from birds is greatly influenced by lighting conditions. Cain and Wilson (1974) reported that the birds given 1h of darkness each day produced a marked decrease in body temperature during that time; there was virtually no activity during 1-h dark phase and increased activity both before and after it. MacLeod *et al.* (1982) found that the pattern of metabolic rate is similar to that of body temperature with a temperature overshoot usually following the dark phase. In laying hens, about 20% of daily heat production is attributable to activity (MacLeod *et al.*, 1982; Boshouwers and Nicaise, 1985). The large difference in HP between the dark and light periods of the day is partly attributed to the difference in activity (MacLeod *et al.*, 1982). The energy expenditure associated with activity has also been studied under different lighting patterns by MacLeod *et al.* (1988). Broiler chickens normally do not eat during darkness, as long as this period does not extend for more than 12 h (Savory, 1979). When a restricted feeding regimen is used, as reported by Koh and MacLeod (1999), HP is at its maximum (peak) at feeding time and tends to decrease, mostly because of increased physical activity of birds. The birds tend to eat most of their food soon after it was given, which would change the distribution of heat increment over the day. However, the birds with free access to food all day, as in the present experiment, are expected to show less pronounced peaks in HP associated with feeding.



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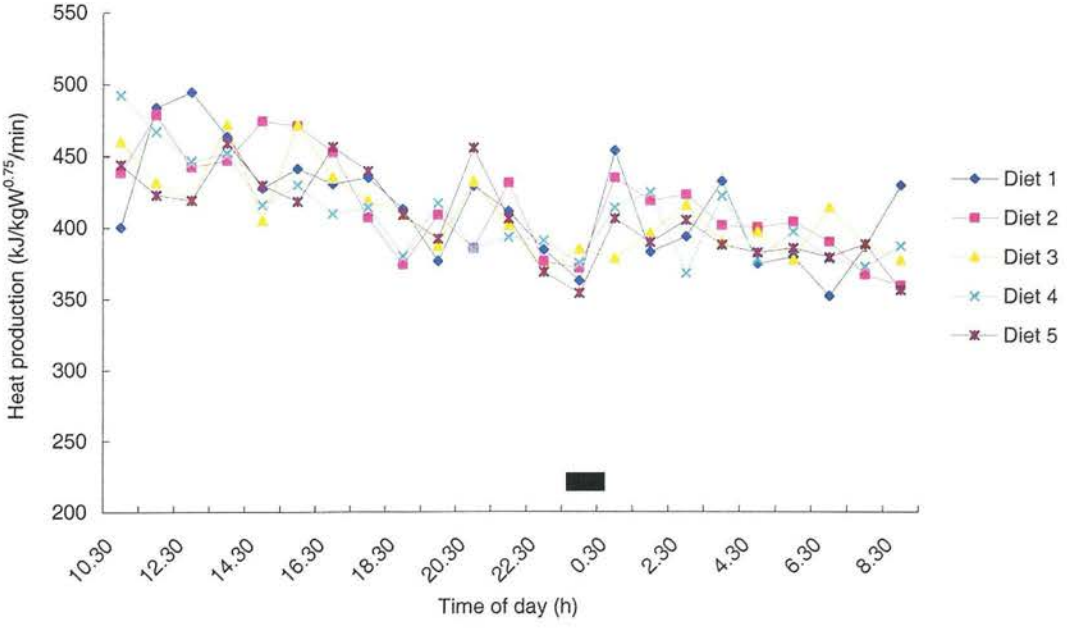
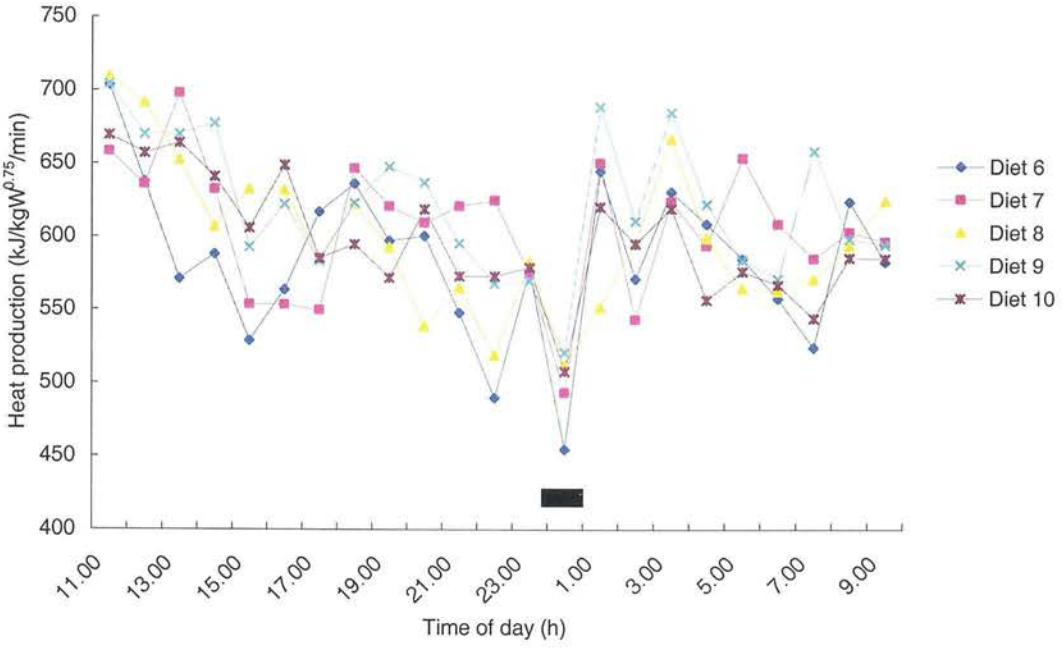


(b)

Figure 1 *Patterns of diurnal variation in the HP rates of fed (a) and fasted (b) birds in experiment 1. The black horizontal bar represent the dark period (1h).*



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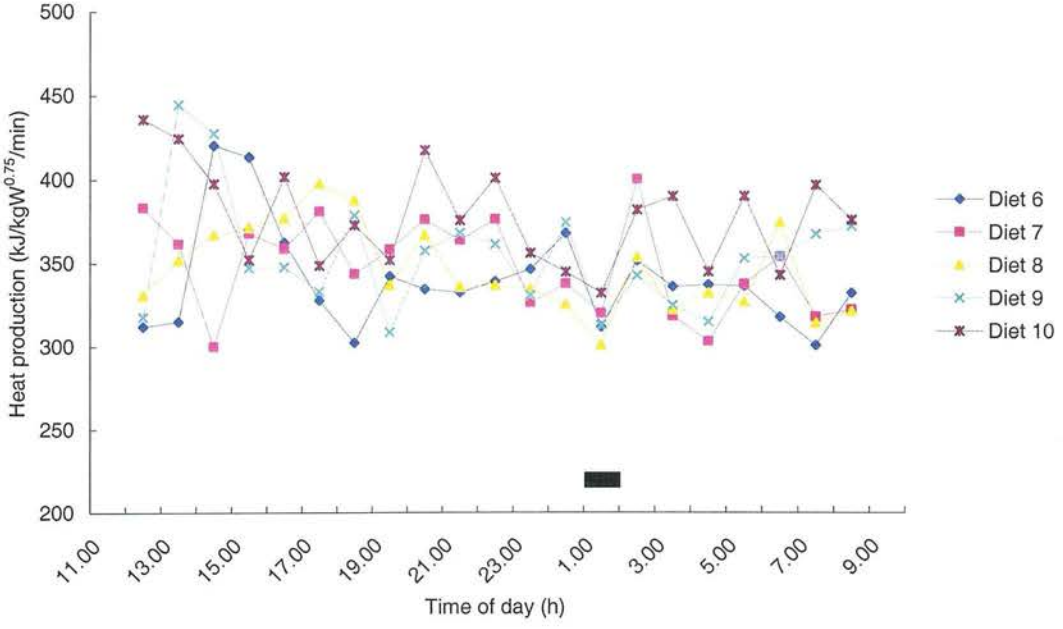
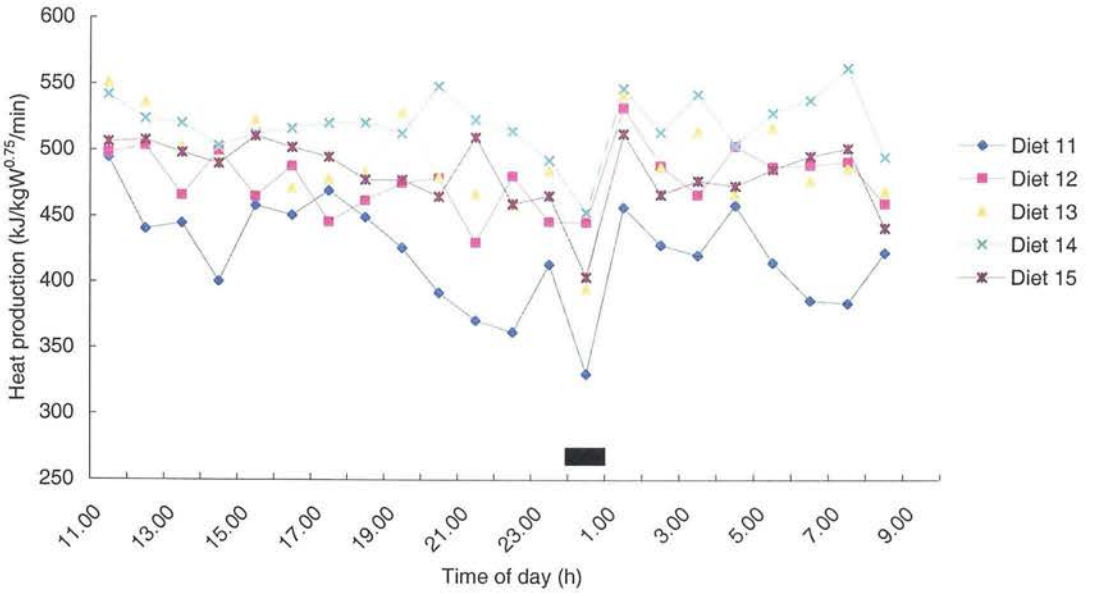


(b)

Figure 2 *Patterns of diurnal variation in the HP rates of fed (a) and fasted (b) birds in experiment 2. The black horizontal bar represent the dark period (1h).*



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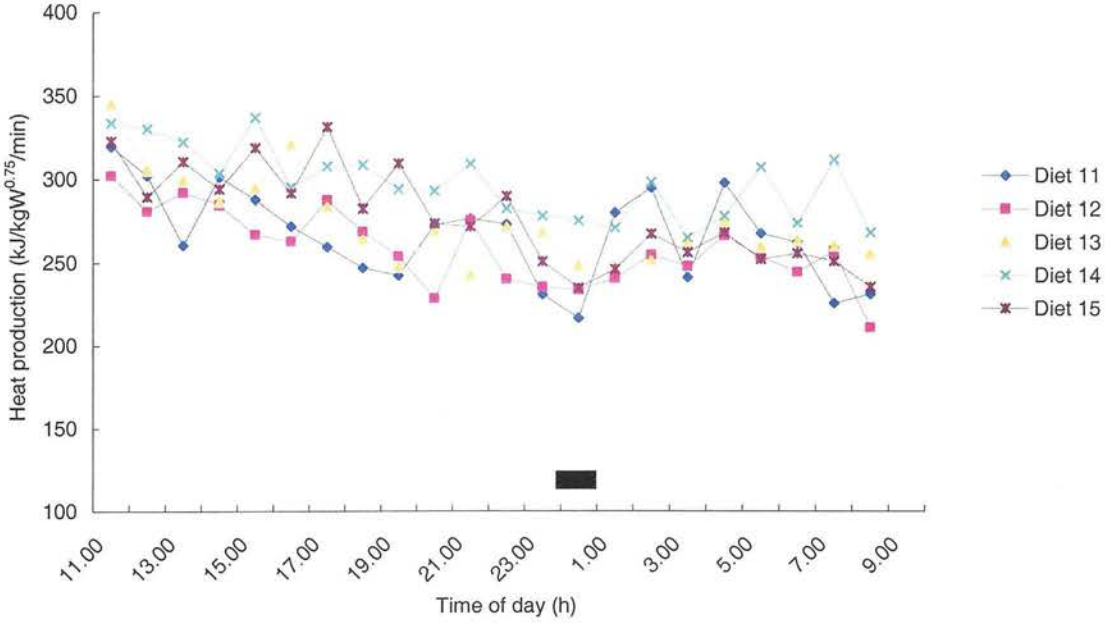

(b)

Figure 3 *Patterns of diurnal variation in the HP rates of fed (a) and fasted (b) birds in experiment 3. The black horizontal bar represent the dark period (1h).*

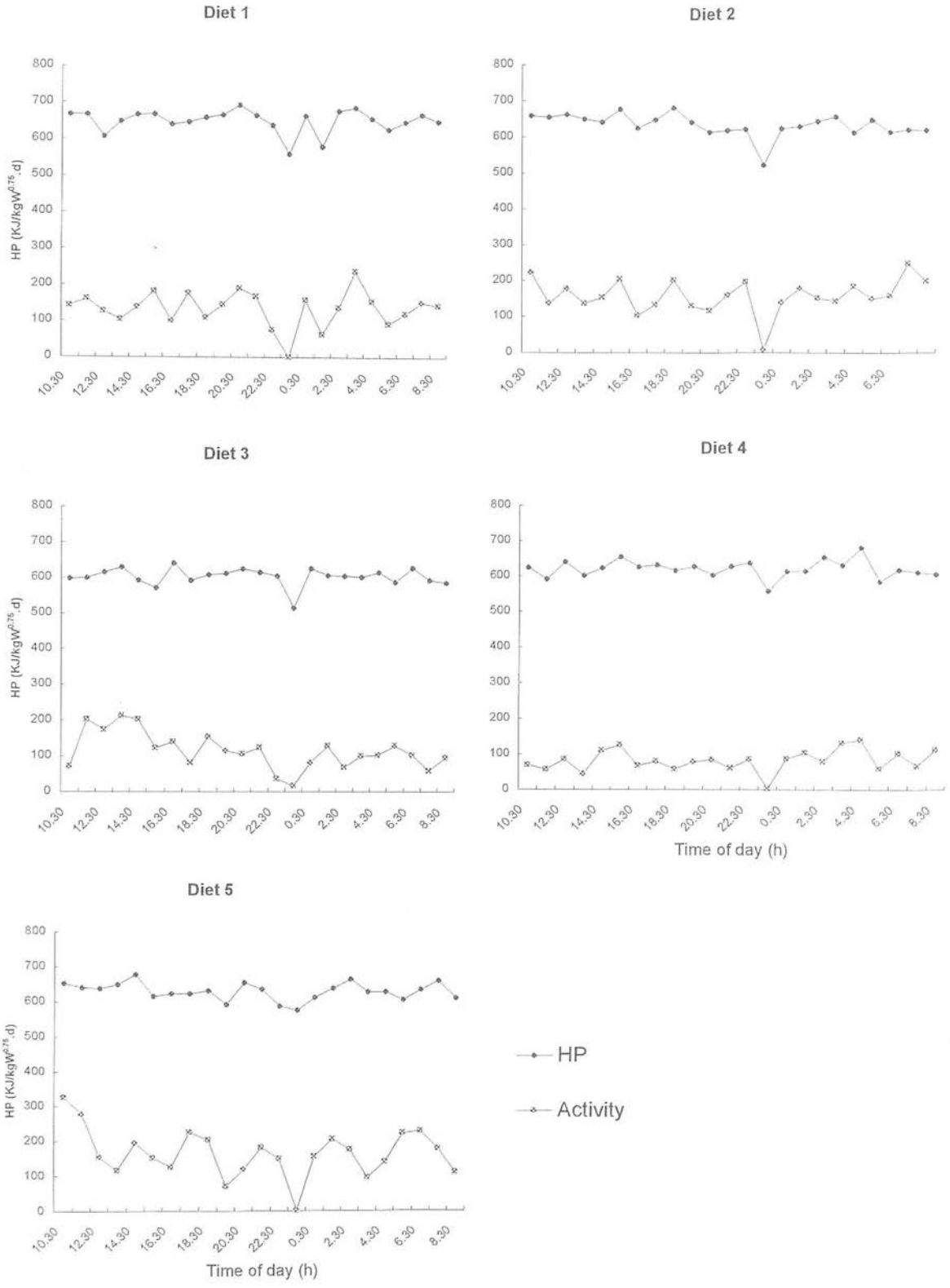

Figure 4 *Diurnal variation in HP rate and physical activity in experiment 1*

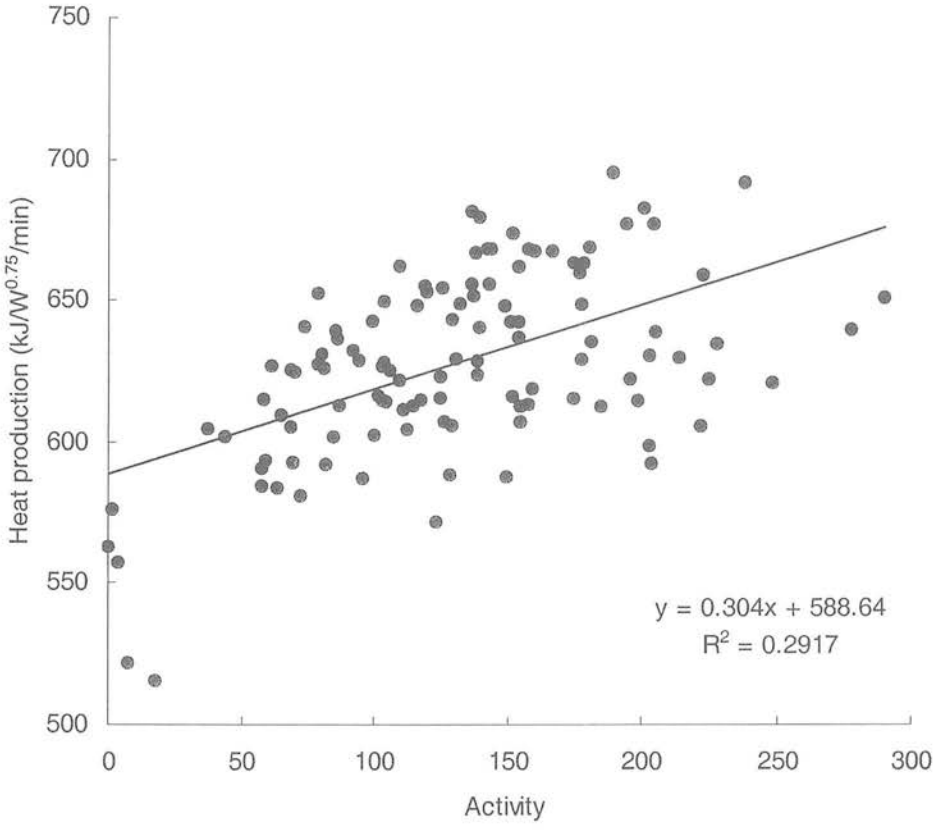

Figure 5 *The relationship between physical activity and heat production in experiment 1*

References

Blaxter, K.L. (1989) *Energy Metabolism*. Cambridge University Press.

Boshauwers, F.M.G. and Nicaise, E. (1985) Automatic gravimetric calorimeter with simultaneous recording of physical activity for poultry. *British Poultry Science*, 26: 531-541.

Cain, J.R. and Wilson, W.O. (1974) The influence of specific environmental parameters on the circadian rhythms of chickens. *Poultry Science*, 53: 1438-1447.

Klasing, K.C. (1998) Energy. In: *Comparative Avian Nutrition*, Wallingford, CAB International.

Koh, K. and MacLeod, M.G. (1999) Circadian variation in heat production and respiratory quotient in growing broilers maintained at different food intakes and ambient temperatures. *British Poultry Science*, 40: 353-356.

MacLeod, M.G., Jewitt, T.R., White, J., Verbrugge, M. and Mitchell, M.A. (1982) The contribution of locomotor activity to energy expenditure in the domestic fowl, in: *Proceedings of the 9th EAAP Symposium on the Energy Metabolism of Farm Animals*, pp. 297-300. (Edit. A. Ekern and F. Sundstol) Aas, Agricultural University of Norway.

MacLeod, M.G., Lundy, H. and Jewitt, T.R. (1985) Heat production by the mature male turkey (*Meleagris gallopavo*): preliminary measurements in an automated, indirect, open-circuit multi-calorimeter system. *British Poultry Science*, 26: 325-33.

MacLeod, M.G., Jewitt, T.R. and Anderson, J.E.M. (1988) Energy expenditure and physical activity in domestic fowl kept on standard and interrupted lighting patterns. *British Poultry Science*, 29:231-244.

Savory, C.J. (1979) Feeding Behavior. In: *Food Intake Regulation in Poultry* pp. 277-323 (Edit. K.N. Boorman and B.M. Freeman) Longmans, Edinburgh, U.K.

PUBLICATIONS ARISING FROM THIS THESIS

J-H. KIM and M.G. MACLEOD (2000) Effects of protein:lysine ratio on energy and nitrogen metabolism in growing broiler chickens. British Poultry Science, 41:693-694.

J.-H. KIM and M.G. MACLEOD (2001) Effects of amino acid balance on energy metabolism in broiler chickens: measurement and modeling. Proceedings of the 15th Symposium on Energy Metabolism in Animals, EAAP Publication No.103: 113-116.

J.-H. KIM and M.G. MACLEOD (2002) Diet Selection on lysine content by growing broiler chickens. British Poultry Science, 43: S37-S39

M.G. MACLEOD, L. MCNEILL and J.-H. KIM (2003) Food intake, weight gain, food conversion ratio, breast muscle weight and abdominal fat weight in chickens fed on diets of varying protein quality. British Poultry Science, 44 : S28-S29.

British Poultry Science, 41:693-694.

Effects of protein:lysine ratio on energy and nitrogen metabolism in growing broiler chickens

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Dietary protein quality influences growth but also affects the environment because of different rates of nitrogen excretion. Reduction in nitrogen excretion and increased efficiency of nitrogen deposition can be obtained by matching the amino acid composition of the diet with amino acid requirements. The conventional expectation is that an imbalanced blend of amino acids must lead to increased energetic costs of catabolism and excretion, accompanied by reduced food conversion efficiency.

Boorman and Ellis (1996) found no adverse effect of protein quality on lysine utilisation and detected some indication of increasing net utilisation with decreased protein quality. MacLeod (1997) examined that there was negative correlation between protein retention per g of lysine consumed and lysine:CP ratio, suggesting that response to a limiting amino acid was improved by the presence of a super-abundance of other amino acids. This experiment was based on diets formulated to have a wide range of protein concentrations but equal concentrations of lysine, the first-limiting amino acid. The detailed relationships among amino acid balance, nitrogen metabolism and energy metabolism were investigated in a computer-controlled chamber calorimetry system.

Male broiler chicks (1d old) from the same commercial line were obtained in batches at 14d intervals and reared to 20d of age on a common diet. At 21d of age, chicks were randomly allocated (in pairs) to calorimeter chambers. The experiment had an acclimatization period of 7 d in which animals were allowed to become accustomed to the diet and calorimeter chamber.

The lysine concentration of each experimental diet was a constant 11g/kg with crude protein (CP) concentration ranging from 180g/kg to 300g/kg (Table 1). The 5 replicate pairs of birds on each diet were allocated according to a 5×5 Latin square design with 5 2-week periods and 5 treatments. Calorimeter chambers were kept at 20°C and on a lighting cycle of 23h light: 1h dark. The experimental diet was given *ad libitum.* The measurements were made from d 28 to d 32. Droppings were collected for 5 d (3d fed and 2d fasted) for nitrogen (N), energy (E) and amino acid analysis.

Table 1. *Specifications of experimental diets*

Diet	1	2	3	4	5
True Metabolisable E (TME) MJ/kg	13.4	13.4	13.4	13.4	13.4
Crude protein (CP) g/kg	180	210	240	270	300
Lysine concentration g/kg	11	11	11	11	11
Lysine : CP ratio	0.061	0.052	0.046	0.041	0.037

There was no effect of CP concentration on food intake (Table 2). However water intake and dry matter excretion were significantly affected by CP concentration. There was no significant effect of amino acid balance on heat production. Heat production was not affected significantly by increasing CP content. N retention did not change significantly as diet protein content increased. N loss in excreta significantly increased with CP content. CP content had highly significant effects on efficiency of N utilisation.

Table 2. *Responses to diet*

Diet	1	2	3	4	5	SED	*P*
Food intake (g / bird. d)	132.1	127.3	126.6	123.7	129.1	4.99	0.568
Wt gain (g / bird. d)	80.6	66.3	73.7	78.6	69.5	9.85	0.589
Heat production (kJ / bird. d)	1037	1021	1038	988	999	33.4	0.491
TME intake (kJ/bird.d)	1870	1918	1824	1844	1763	64.5	0.245
E retention (kJ / bird.d)	725	800	680	754	654	45.7	0.056
N intake (g / bird.d)	4.10	4.18	5.29	5.90	6.18	0.212	<0.001
N retention (g / bird.d)	2.68	2.43	2.60	2.61	2.60	0.147	0.541
N loss (g / bird.d)	1.41	1.75	2.68	3.29	3.59	0.168	<0.001
Efficiency of N retention	0.66	0.58	0.49	0.44	0.42	0.0219	<0.001
CP retention	16.77	15.19	16.28	16.34	16.22	0.918	0.541
E retention as protein	398	360	386	387	384	21.76	0.541
E retention as fat	327.7	440.3	294.3	366.5	269.8	30.84	0.001
Retention of fat by weight	8.36	11.23	7.51	9.35	6.88	0.787	0.001

The results suggested that protein synthesis remained almost constant on diets with wide range of protein content but constant lysine concentration. Amino acid balance did not have a significant effect on heat production, despite significant changes in rate of N excretion. Reduction in dietary CP level has a beneficial effect in terms of reducing N excretion.

BOORMAN, K.N. & ELLIS, G.M. (1996) The maximum nutritional response to poor-quality protein and amino acid utilisation. British Poultry Science, **37**: 145-156

MACLEOD, M.G. (1997) Effect of amino acid balance and energy: protein ratio on energy and nitrogen metabolism in male broiler chickens. British Poultry Science, **38**: 405-411

Proceedings of the 15th Symposium on Energy Metabolism in Animals, EAAP Publication No.103: 113-116.

Effects of amino acid balance on energy and nitrogen metabolism in broiler chickens: measurement and modelling

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Summary

Experiments and computer simulations were performed to test the assumption that imbalanced dietary amino acid mixtures must lead to increased heat production. The first experiment described was based on diets formulated to have a wide range of crude protein (CP) concentrations but a fixed concentration of lysine, formulated to be the first-limiting amino acid. In the second (converse) experiment, lysine concentration was varied over a wide range while CP content was kept constant. The detailed relationships among amino acid balance, nitrogen (N) metabolism and energy (E) metabolism were investigated in a computer-controlled chamber calorimetry system. The results of both experiments were compared with the predictions of a computerised simulation model of E metabolism. In experiment 1, with constant lysine and varying CP, there was a 75% increase in N intake as CP concentration increased. This led to a 150% increase in N excretion, with no significant change in heat production. Simulated heat production agreed with the empirically determined results in not showing a trend with dietary CP. In experiment 2, with varying lysine but constant CP, there was a 3-fold difference in daily weight gain between the lowest and highest lysine diets. Heat production per bird increased significantly with dietary lysine concentration. There was still an effect when heat production was adjusted for body weight differences, but it failed to maintain statistical significance. Simulated heat production results agreed in showing little effect of varying lysine concentration and growth rate on H.

Keywords: amino acid balance, broiler chicken, heat production, modelling, nitrogen metabolism

Introduction

The conventional expectation has been that a balanced blend of amino acids must lead to decreased energetic costs of catabolism and excretion (Baldini, 1961; Guillaume & Summers, 1970). MacLeod (1997) found, however, that heat production was closely correlated with rate of protein accretion (which in turn was strongly associated with the intake of the first-limiting amino acid) and was affected little by total crude protein (CP) intake. However, the experiment of MacLeod (1997) used a rather heterogeneous series of diets and it was felt that more linear series of diets would lead to more secure

conclusions. The first experiment described in this paper was, therefore, based on diets formulated to have a wide range of linearly varying CP concentration but equal concentrations of lysine, which was fixed as the first-limiting amino acid. The hypothesis being tested was that growth rate would be fixed by lysine concentration and that the energy cost of nitrogen excretion would therefore vary with excess N intake. The second experiment tested the converse, with diets of constant CP content but linearly varying lysine concentrations, with lysine again fixed as the first-limiting amino acid. The hypothesis being tested here was that heat production would vary with growth rate (specifically protein accretion rate), which would itself be controlled by dietary lysine concentration. The experimental results were compared with simulations using the mechanistically based model of energy metabolism described by MacLeod (1994, 1998).

Materials and methods

In both experiments, male broiler chicks (1 d old) from the same commercial line were obtained in batches at 14 d intervals (so that they were of identical age at the time of measurement) and reared to 20d of age on a common starter diet. At 21 d of age, chicks were randomly allocated (in pairs) to calorimeter chambers (Lundy *et al.*, 1978). The experiments had an acclimatisation period of 7 d in which animals were allowed to become accustomed to the diet and calorimeter chamber. The 5 replicate pairs of birds on each diet were allocated according to a 5×5 Latin square design with five 2-week measurement periods and 5 treatments. The E and N metabolism measurements were made continuously from d 28 to d 32 . In experiment 1, the lysine concentration of each experimental diet was a constant 11g/kg, with CP concentration ranging from 180 g/kg to 300 g/kg and TME concentration constant at 13.4 MJ/kg (Table 1). In experiment 2, lysine concentration ranged from 6 to 14 g/kg at a constant CP concentration of 300 g/kg and constant TME concentration of 13.4 MJ/kg (Table 2). In both experiments, other amino acids were maintained at concentrations 1.5 times the published recommendations, to ensure that no other amino acid became limiting. Both diets were pelleted. Food and water were available *ad libitum*. Chamber temperature was 20° C and the birds were kept on a 23L:1D lighting cycle. Statistical analysis was by analysis of variance, including diet, column (chamber) and row (measurement period) in the model. The simulations were done using the mean bird weight at time of measurement (1150 g) and the mean food intake (128 g/d).

Table 1. Specifications of experimental diets for Experiment 1.

Diet	1	2	3	4	5
True metabolisable energy (TME) MJ/kg	13.4	13.4	13.4	13.4	13.4
Crude protein (CP) g/kg	180	210	240	270	300
Lysine concentration g/kg	11	11	11	11	11
Lysine : CP ratio	0.061	0.052	0.046	0.041	0.037

Table 2. Specifications of experimental diets for Experiment 2.

Diet	6	7	8	9	10
True metabolisable energy (TME) MJ/kg	13.4	13.4	13.4	13.4	13.4
Crude protein (CP) g/kg	300	300	300	300	300
Lysine concentration g/kg	6	8	10	12	14
Lysine : CP ratio	0.020	0.027	0.033	0.040	0.047

Results

Experiment 1: constant lysine, varying CP

There was no effect of CP concentration on food intake (Table 3). This meant that there was a significant increase in N intake as CP concentration increased; the increase was by about 75% between the lowest and highest CP diets. However, N retention did not change significantly, because of the constant dietary lysine concentration. This led to an increase by about 150% in N excretion. Despite this very large change in N excretion, there was no significant change in heat production. Simulated heat production agreed with the empirically determined results in not showing a trend with dietary CP. The simulation results can not be given a standard error since the model is not stochastic.

Table 3. Responses to diet, experiment 1

Diet	1	2	3	4	5	SED	*P*
Food intake (g/bird. d)	132.1	127.3	126.6	123.7	129.1	4.99	NS
Food intake(g/kg$W^{0.75}$.d)	119.8	113.4	112.7	111.6	117.0	3.99	NS
E retention (kJ/bird.d)	725	800	680	754	654	45.7	0.05
E retention (kJ/kg$W^{0.75}$.d)	657	712	604	680	593	37.2	<0.05
N intake (g/bird.d)	4.10	4.18	5.29	5.90	6.18	0.212	<0.001
N intake (g/kg$W^{0.75}$.d)	3.17	3.72	4.72	5.32	5.61	0.178	<0.001
N retention (g/bird.d)	2.68	2.43	2.60	2.61	2.60	0.147	NS
N retention (g/kg$W^{0.75}$.d)	2.44	2.16	2.32	2.36	2.35	0.116	NS
N loss (g/bird.d)	1.41	1.75	2.68	3.29	3.59	0.168	<0.001
N loss (g/kg$W^{0.75}$.d)	1.28	1.56	2.40	2.97	3.26	0.154	<0.001
Efficiency of N retention	0.66	0.58	0.49	0.44	0.42	0.022	<0.001
Heat production (H, kJ/bird.d)	1037	1021	1038	988	999	33.4	NS
H (kJ/g$W^{0.75}$.d)	940	909	928	891	902	26.2	NS
Simulated H (kJ/bird.d)	977	977	970	978	979	-	-

Experiment 2: varying lysine, constant CP

There was a 3-fold range in daily weight gain with variation in dietary lysine concentration (Table 4). Heat production per bird increased significantly with rate of growth and therefore with dietary lysine content. A trend remained when heat production was adjusted for body weight differences (kg$W^{0.75}$), but it was no longer statistically significant. Simulated H results also showed little effect of varying lysine

concentration and growth rate on H.

Table 4. Responses to diet, experiment 2.

Diet	6	7	8	9	10	SED	*P*
Food intake (g/bird.d)	88	107	104	133	118	11.9	<0.05
Food intake (g/kgW $^{0.75}$.d)	96	105	100	116	107	8.0	NS
TME intake (kJ/bird.d)	1174	1431	1394	1780	1576	159.4	<0.05
TME intake (kJ/kgW $^{0.75}$.d)	1287	1400	1338	1558	1435	107.6	NS
Wt gain (g/bird.d)	31.3	53.6	67.4	95.1	85.3	9.06	<0.001
E retention (kJ/bird.d)	417	541	508	766	636	108.1	0.06
E retention (kJ/kgW $^{0.75}$.d)	453	532	482	672	578	91.1	NS
Heat production (H, kJ/bird.d)	757	890	885	1013	940	66.2	<0.05
H (kJ/kgW $^{0.75}$.d)	832	868	849	890	860	37.6	NS
Simulated H (kJ/bird.d)	1049	1002	973	979	985	-	-

Discussion

The results suggest that the effects both of protein synthesis and of imbalanced amino acid mixtures on whole-animal heat production can be relatively small. There was no detectable relationship between heat production and rate of N excretion despite the attainment of a 2.5-fold range in N loss. There was a significant relationship between H and dietary lysine concentration but this was partly due to greater body weight on the higher lysine concentrations and the relationship became less significant when H was adjusted for body weight. The resultant regression between H and lysine intake gave less than half the gradient determined by MacLeod (1997), in an experiment using a more diverse range of diets. This was despite a significant correlation between body weight gain and lysine intake (r=0.77; P<0.001).

The relative energy costs of protein accretion and N excretion can be compared in terms of ATP breakdown and synthesis. The energy cost of incorporating an amino acid molecule into a protein is about 4 mol ATP (Schulz, 1978). Excretion, as uric acid, of the N resulting from catabolism of the amino acid uses 6 mol ATP/g.atom N. Excretion of the N from amino acids therefore costs 6 mol ATP/mol amino acid for most amino acids (which contain 1 N) but as much as 18 for histidine (which contains 3 N). However, the cost of uric acid synthesis is more than offset by the energy resulting from oxidation of the amino acid molecule. The ratio of ATP yield (from oxidation) to ATP utilisation for uric acid synthesis is 68:32 (mean of major dietary amino acids). The positive balance of ATP from oxidised amino acids is potentially available to spare the oxidation of other substrates. This was confirmed by the simulation results; where there was a high protein content with low concentrations of the first-limiting amino acid, the simulation programme attributed the energy resulting from oxidation and excretion of the excess amino acids to an increased maintenance heat production. At temperatures below thermoneutral, any additional energy available may also be used for thermoregulation, although the experiment of MacLeod (1997) was also performed at 20° C and

demonstrated a wide range of heat productions in relation to lysine intake and rate of protein accretion. However, to prevent the masking of dietary effects by thermoregulatory demands, further similar experiments are planned at 30° C. At higher temperatures, it is possible that a further interaction may occur: any additional thermogenic effect of the diet may lead to reduced food intake if the bird is unable to dissipate additional heat.

Acknowledgments

J-H. Kim was funded by the Korean Collaboration Centre and Roslin Institute. The contribution of M.G. MacLeod was funded by the Ministry of Agriculture, Fisheries and Food (MAFF).

References

Baldini, J.T., 1961. The effect of dietary deficiency on the energy metabolism of the chick. Poult. Sci. 40: 1177-1183.

Guillaume, J. & J.D. Summers, 1970. Influence of amino acid excess on energy utilisation in the growing chick.. Can. J. An. Sci. 50: 355-362.

Lundy, H., M.G. MacLeod, M.G. & T.R. Jewitt, 1978. An automated multi-calorimeter system: preliminary experiments on laying hens. Br. Poult. Sci. 19: 173-186.

MacLeod, M.G., 1994. Dietary energy utilisation: prediction by computer simulation of energy metabolism. Proc. 13th Symp. Energy Metab. Farm Animals. Mojacar, Spain. 237-240.

MacLeod, M.G., 1997. Effect of amino acid balance and energy:protein ratio on energy and nitrogen metabolism in male broiler chickens. Br. Poult. Sci. 38: 405-411.

MacLeod, M.G., 1998. Dietary energy utilisation: sensitivity of a net energy model to varying estimates of food intake, maintenance requirement and protein deposition. Proc. 14th Symp.Energy Metab. Farm Animals. Newcastle, County Down. 323-326.

Schulz, A.R., 1978. Simulation of energy metabolism in the simple-stomached animal. Br. J. Nutr., 39: 235-254.

British Poultry Science, 43: S37-S39

Diet selection on lysine content by growing broiler chickens

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This experiment was carried out in connection with work on the effects of amino acid balance on energy metabolism (Kim and MacLeod, 2001). Free-choice feeding was performed with diets containing the same amount of crude protein but with a range of lysine concentrations. The diets were designed so that lysine was the first-limiting amino acid. The objective was to investigate the sensitivity of the broiler chicken to dietary amino acid balance, especially in relation to lysine concentration.

Forty-eight male broiler chicks were reared in brooders for 20 d after hatch on a common starter diet. At 21 d of age, they were randomly allocated (in pairs) to cages fitted with two feeding troughs and a water container. Three diets with different lysine concentrations were formulated. The lysine concentrations were 6 g/kg (low), 10 g/kg (moderate; NRC (1994) recommendation for 3-6 wk old broiler) and 14 g/kg (high) respectively. Other amino acids were maintained at concentrations 1.5 times the published recommendations, to ensure that no other amino acid became limiting. The diets were made by adding supplements to the basal diet to minimise palatability effects of altering major feed ingredients. As supplemental lysine was removed, it was replaced with 2X its weight of glutamic acid to keep diets isonitrogenous. Birds were divided into 4 treatment groups. Group A was offered the choice between the low lysine diet and the moderate lysine diet. Group B was offered the low and high lysine diets. Group C was given the moderate and high lysine diets. Group D had only the moderate lysine diet, to act as the control group, but the food was given in two separate troughs. All diets contained 300 g/kg of crude protein and 13.4 MJ TME/kg. Two days were allowed for the birds to become accustomed to the experimental set-up. Food and water intake and body weight were measured daily thereafter, for 10 days. The data were analysed by one sample t-test and analysis of variance.

Throughout the experimental period, groups A and B showed a very similar degree of preference for the higher of the two lysine concentrations which they were offered (Table 1). The selection became more significant with time. Both groups consumed about 2.5 times as much of the moderate and high lysine diets as of the low lysine diet. In group B, this led to a lysine intake equal to that of the control group (D). In the case of group C, the birds did not show a preference between moderate-lysine and high-lysine diets, but this still led to a higher lysine intake than that of the control group (D). Group A and B, which had a low lysine diet included in their choice, showed lower total food and water intake and growth rate than the groups offered the high lysine diet (Table 2). Growth rate reflected total lysine intake (Figure).

Table 1. *Selection between low, moderate and high lysine diets.*

Group	A		B		C		D	
Lysine content	Low[1]	Mod [2]	Low	High [3]	Mod	High	Mod	Mod
Proportions of intake d1-d5	0.436	0.564	0.465	0.535	0.485	0.515	0.495	0.505
Proportions of intake d6-d10	0.300	0.700	0.281	0.719	0.521	0.479	0.493	0.507
Proportions of intake d1-d10	0.357	0.643	0.362	0.638	0.506	0.494	0.494	0.506
Differences from equality in proportions of intake d1-d5	0.064 (P=0.058)		0.036 (P=0.015)		0.015 (P=0.245)		0.005 (P=0.807)	
Differences from equality in proportions of intake d6-d10	0.201 (P=0.002)		0.219 (P<0.001)		-0.021 (P=0.238)		0.007 (P=0.785)	
Differences from equality in proportions of intake d1-d10	0.143 (P=0.002)		0.138 (P=0.001)		-0.006 (P=0.064)		0.006 (P=0.767)	

[1] Low, 6g/kg lysine; [2] Moderate, 10g/kg lysine (NRC recommendation); [3] High, 14g/kg lysine.

* Statistical significance of the choice was tested by one-sample t-test of the null hypothesis that the birds ate equal amounts of the two diets.

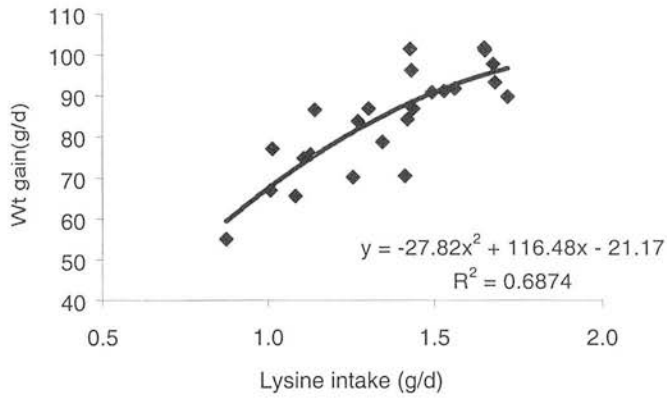

Figure. *Relationship between weight gain and lysine intake, including all diet choices.*

Table 2. *Total food intake, water intake and lysine intake (d1-d10).*

Group	A	B	C	D	SED	P
Total food intake (g/d)	123.7	125.1	136.3	135.2	6.45	0.003
Water intake (ml/d)	246.3	259.0	294.0	252.2	13.49	0.003
Total lysine intake (g/d)	1.063	1.387	1.633	1.352	0.079	<0.001

Although the birds in group A showed some preference for the moderate-lysine diet, the preference was insufficient to produce as high a growth rate as in control group D. In this experiment, broiler chickens showed an ability to select between different concentrations of lysine but the choice did not always lead to the lysine concentration that would produce maximal growth rate.

KIM, J.-H. and MACLEOD, M.G. (2001). Effects of amino acid balance on energy metabolism in broiler chickens: measurement and modeling. *Proceedings of the 15th Symposium on Energy Metabolism in Animals*, EAAP Publication No.**103**: 113-116.

Food intake, weight gain, food conversion ratio, breast muscle weight and abdominal fat weight in broiler chickens fed on diets of varying protein quality

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An ideal protein diet is one that provides a mixture of amino acids as close as possible to the mixture required by the bird. This can be approached most closely by supplementation with the most-limiting amino acids. In this experiment an "ideal protein" diet was formulated, by linear programming, to provide NRC requirements of amino acids at the minimum protein content allowed by supplementation of commonly-used raw materials with lysine, methionine, threonine, tryptophan and arginine. At the other extreme, an isoenergetic high protein diet was formulated to provide the same amino acid intakes without supplementation. Two intermediate diets were formulated by linear interpolation (Table 1). It is sometimes stated that fat content is higher on an ideal protein diet and that the maximum response to the first-limiting amino acid is not attained on a high protein diet. However, before these statements can be safely assessed, it is important to make an adjustment for the difference in digestibility between the supplemental (synthetic, crystalline) amino acids and those in the natural raw materials. Total lysine content in the highest protein diet was, therefore, increased by 5% to allow for the greater digestibility of the supplement in the "ideal" and intermediate diets. The analogous calculation for methionine + cystine led to increasing total methionine by 9% in the "high protein" diet. In commercial formulation, the correction could be made by formulating on digestible amino acids. The experiment was designed in randomised blocks with 5 replicate pens (1·5 m × 2·4 m) of 20 male Ross 308 broilers on each diet.

Body weight and cumulative food intake were measured at 21-d intervals. At the end of the 42 d growing period, two birds of near-mean weight were sampled from each pen for dissection (breast meat yield and fat pad weight). The results were subjected to analysis of variance. Food intake (Table 2) was very similar between "ideal protein" and "high protein" diets. There was a tendency for intake on the intermediate diets to be slightly lower but this was statistically significant only between d 1 and d 21. Weight gain was near commercial target. It did not differ significantly between ideal and imbalanced diets and the slightly lower weight gain

Table 1. *Ideal and high protein diets. The two additional diets were linearly intermediate in all ingredients. Units are g/kg unless otherwise indicated*

	Diet 1 (ideal protein)	Diet 2	Diet 3	Diet 4 (high protein)
Wheat meal	665·0	610·9	556·8	502·8
Soya (480 g CP/kg)	160·5	177·7	194·9	212·0
Maize gluten 600 g CP/kg	40·3	76·9	113·4	150·0
Fish meal	29·00	38·54	48·08	57·62
Soya oil	50·8	47·0	43·2	39·4
Dicalcium phosphate	14·00	12·25	10·49	8·74
Limestone flour	8·57	8·16	7·75	7·34
Pellet binder	10·0	10·0	10·0	10·0
Choline chloride	0·30	0·30	0·30	0·30
Salt (sodium chloride)	3·42	3·17	3·04	2·80
Vitamins and minerals	5·0	5·0	5·0	5·0
Titanium dioxide	4·0	4·0	4·0	4·0
Supplementation				
L-arginine	1·85	1·24	0·62	0·00
L-lysine hydrochloride	3·46	2·30	1·15	0·00
DL-methionine	2·52	1·68	0·84	0·00
L-threonine	1·28	0·86	0·43	0·00
Analysis				
TME_N (MJ/kg)	13·4	13·4	13·4	13·4
Crude protein	206	235	265	294
Lysine	11·00	11·20	11·40	11·60
Methionine+cystine	9·00	9·35	9·69	10·04
Threonine	8·00	8·90	9·81	10·71
Tryptophan	2·14	2·37	2·61	2·84
Arginine	12·50	13·28	14·07	14·85

Table 2. *Cumulative food intake, cumulative weight gain, food conversion efficiency (FCE), breast muscle weight and abdominal fat pad weight in broiler chickens fed on diets of varying protein quality*

	Diet 1	Diet 2	Diet 3	Diet 4	SEM	LSD	P
Feed (g) d 0–d 21	1077	996	983	1097	30·5	94·1	0·050
Feed (g) d 21–d 42	3225	3125	3130	3157	65·3	201·3	0·692
Feed (g) d 0–d 42	4302	4121	4113	4254	74·1	228·4	0·232
Gain (g) d 0–d 21	751	701	698	779	21·4	30·2	0·054
Gain (g) d 21–d 42	1809	1811	1776	1794	27·0	83·2	0·781
Gain (g) d 0–d 42	2560	2512	2474	2573	33·9	104·6	0·203
FCE d 0–d 21	0·69	0·70	0·71	0·71	0·0115	0·0355	0·796
FCE d 21–d 42	0·56	0·57	0·57	0·57	0·0082	0·0252	0·474
FCE d 0–d 42	0·60	0·61	0·60	0·61	0·0071	0·0219	0·519
Breast weight (g)	455	406	439	404	18·93	54·61	0·170
Breast proportion	0·169	0·156	0·163	0·152	0·0066	0·0190	0·279
Fat pad weight (g)	35·5	35·1	39·9	28·5	3·34	9·63	0·137
Fat proportion	0·0133	0·0135	0·0148	0·0106	0·00128	0·00369	0·146

on the intermediate diets did not reach significance even in the first half of the experiment. Food conversion efficiency (FCE, weight gain/food intake) was similar in all diets. Breast muscle weight, in both absolute and relative terms, was highest on the ideal protein diets but this effect did not attain statistical significance. Fat pad weight did not differ significantly between diets but tended to be lowest on the "high protein" diet. In general terms, the results of this experiment confirmed those of Kim and MacLeod (2001), in that reducing total crude protein content while maintaining the intake of essential amino acids reduced nitrogen losses without showing any significant detrimental effects on growth rate, body composition or breast meat yield.

This work was supported by DEFRA.

REFERENCES

KIM, J.-H. & MACLEOD, M.G. (2001) Effects of amino acid balance on energy metabolism in broiler chickens: measurement and modelling. *Proceedings of the 15th Symposium on Energy Metabolism in Animals*, EAAP Publication No. 103: 113–116.